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Abstract Book

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ORAL PRESENTATION

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Targeting HOX expression through XPO1 inhibition in NPM1-mutated acute myeloid leukemia

Background and hypothesis

NPM1-mutated acute myeloid leukemia (AML) is the most frequent AML subtype in adults accounting for one third of newly diagnosed cases. NPM1 mutations lead to the generation of a nuclear export signal (NES), which result in a significant interaction with the nuclear exporter XPO1 and the accumulation of mutant NPM1 (NPM1c) in the cytoplasm of AML cells. One of the features of NPM1-mutated AML is the high expression of the homeobox (HOX) genes, which are known to be necessary for the maintenance of the leukemic state. As XPO1 has been previously reported to be bound to chromatin, we reasoned that NPM1c could be found not only in the cytoplasm, but also on chromatin, where it may facilitate the expression of their targets, including HOX. We also hypothesized that pharmacologic targeting of the XPO1-NPM1c interaction by XPO1 inhibitors would lead to loss of HOX expression and differentiation of AML cells in vitro and in vivo.

Aims

1) determining whether NPM1c and XPO1 are enriched at HOX and whether they facilitate their expression; 2) optimizing pharmacologic inhibition of XPO1 by the XPO1 inhibitor Eltanexor (Elta); 3) determining the preliminary clinical safety and efficacy of Elta in relapsed/refractory NPM1-mutated AML patients.

Experimental design

1) ChIP sequencing against NPM1 and XPO1 performed in an NPM1-mutated cell line (OCI-AML3) at baseline and following endogenous NPM1c degradation through a CRISPR-engineered degron system as well as in primary samples; 2) treatment of cell lines, primary samples and two NPM1-mutated patient xenograft models (PDX) with two different XPO1 inhibitors followed by ChIP-sequencing transcriptome profiling and flow cytometry for differentiation and cell cycle analysis; 3) design, approval and conduction of a pilot phase 2 study recruiting 10 patients with relapsed or refractory NPM1-mutated AML.

Results

ChIP-sequencing for NPM1 and XPO1 performed in the degron cell line enabled the identification of a small set of high-confidence genes, including HOXA and HOXB genes, with strong NPM1c enrichment (33 genes, referred as to NPM1c targets). Importantly, the expression of NPM1c targets was lost as soon as 15 minutes following the beginning of NPM1c degradation. Loss of NPM1c resulted in only minor loss of XPO1 at the DNA level, suggesting that XPO1 is responsible for recruiting NPM1c at their loci through the NES. Pharmacologic inhibition of XPO1 with two different XPO1 inhibitors resulted in the release of NPM1c from chromatin, immediate HOX downregulation, differentiation in vitro. Next, two PDX models were treated with two different XPO1 inhibitors (Selinexor and Elta), administered according to their known toxicity profiles in humans. 5 days/week Elta for 28 consecutive days was the most effective regimen, which caused differentiation, loss of AML engraftment and prolonged survival of PDXs. Finally, we recently started an academic clinical study that will treat 10 NPM1-mutated AML patients with 5 days/week 20 mg Elta for cycles

of 28 days. To date, one patient has been treated and the results will be discussed at the meeting.

Conclusions

Targeting HOX expression by XPO1 inhibition is a promising therapeutic approach in NPM1-mutated AML.

Matteo Calvaresi

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NanoPhages: Engineered Viral Vectors for Receptor Targeted Photodynamic Therapy

Background and hypothesis

Photodynamic therapy (PDT) is a clinically approved, minimally invasive procedure for cancer treatments. In PDT, a compound with photosensitizing properties (sensitizer, PS) upon activation by light, generates reactive oxygen species (ROS), responsible for cytotoxicity in cancer cells. The success of PDT is usually limited by: i) poor water solubility and low biocompatibility of the sensitizers ii) lack of selective accumulation of the PS at cancer cells, resulting in unwanted phototoxicity.

Phages are naturally occurring viruses that develop high selectivity for certain bacteria, while they are inactive against eukaryotic cells. The great ease of manipulation of the phage genome, allows to display peptides/antibodies on the phage surface, retargeting them to any type of cell. At the same time hundreds of therapeutic and imaging agents can be conjugated onto the phage capsid, providing a robust and flexible platform for anticancer approaches.

Aims

NanoPhage aims to create an innovative phage-based phototheranostic platform for receptor targeted PDT and imaging.

We developed synthetic methodologies to chemically functionalize the phage capsid with sensitizers/imaging tags and direct this viral vector selectively to cancer cells via phage display of targeting-predatory biomolecules. We targeted HER2 and EGFR receptors, because they are frequently over-expressed in various cancers.

Experimental design

An orthogonal nanoarchitectonics approach (genetic/chemical) was developed to engineer M13 bacteriophages as phototheranostic platforms.

M13 was genetically refactored to display on the phage tip a peptide, a nanobody or a single chain antibody able to bind epidermal growth factor receptors such as EGFR or HER2. Using an orthogonal approach to the genetic display, the refactored phages were then chemically modified, conjugating hundreds of sensitizers on the capsid surface.

Results

Flow cytometry and confocal microscopy experiments demonstrated the efficient retargeting of the phages to cancer cells overexpressing EGFR/HER2. Upon internalization, the phage conjugates generated intracellularly reactive oxygen species, activated by an ultralow intensity light irradiation. The killing activity of cancer cells was observed at picomolar concentration of the phage vector. The mechanism of cell killing was investigated. The phage-bioconjugates showed a high permeation/phototoxicity also into 3D spheroids and ex vivo samples. The nanosafety of the phototheranostic platform and its imaging/therapeutic performances were preliminary evaluated in vivo using Hydra vulgaris as a model organism.

Conclusions

NanoPhage project developed a modular phage carrier for targeted cancer treatment and imaging, promoting the development of a theranostic platform able to simultaneously detect, image and kill cancer cells.

NanoPhage combined the advantages of immunoconjugates anticancer therapies (specific, highly effective, minimally toxic) with the possibility to load orthogonally hundreds of effectors in the viral platform. Soft and penetrating irradiation sources were used to activate the NanoPhages. A double-targeting therapy was developed, exploiting the selectivity of the NanoPhages and the possibility to have a focused irradiation at the desired site of action, lowering the collateral damage to healthy tissues.

Michelangelo Campanella

Università degli Studi di Padova, Padova

Mitochondria form contacts with the nucleus to couple prosurvival retrograde response in breast cancer cells

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Background and hypothesis

Mitochondria actively take part in the homeostasis of mammalian cells. In response to endogenous or exogenous stressors, mitochondria retro-communicate with the nucleus to induce wide-ranging cytoprotective effects that sustain uncontrolled growth. This process, which goes under the name of mitochondrial retrograde response (MRR), is acknowledged to be driven by the deregulation of Reactive Oxygen Species (ROS), Ca2+ signalling and energy defects that promote nuclear stabilization of transcription factors (e.g. NF-κB).

We uncovered that the physical coupling between mitochondria and the nucleus facilitates this pro-survival route of communication. Based on this, we hypothesised whether these new sites of intracellular contact might have a part in the pathogenesis of hormone-derived cancers, such as those of the mammary gland, representing therefore a target to bio-mark and overcome resistance to endocrine-based therapy (ET).

Aims

Using breast cancer cells (BCCs) we assessed the juxtaposition of mitochondria and the nucleus along with the molecular axis of signalling between the two organelles in the evolution of the malignancies and susceptibility to ET. This was done following molecular and pharmacological modulation of the Outer Mitochondrial Membrane (OMM) Protein TSPO which binds and traffics cholesterol whose contribution to MRR signalling was also assessed.

Experimental design

We enrolled methods of advanced cell imaging together with protocols of structural and ultrastructural analysis we assessed the juxtaposition of mitochondria and the nucleus (i), the molecular axis of signalling between the two organelles (ii) in the evolution of the malignancies and susceptibility to ET. This was done in breast cancer cells (BCCs) following molecular and pharmacological modulation of the Outer Mitochondrial Membrane (OMM) Protein TSPO which binds and traffics the lipid cholesterol whose contribution to MRR was also assessed.

Results

TSPO was proven to be required for the formation of contacts between mitochondria and the nucleus, which we termed as Nucleus nucleus-associated mitochondria (NAM). Upscaled in BCCs which develop resistance to ET, TSPO drives an increment in the frequency of NAM which are positively correlated with the aggressiveness of the lesions and redistribution of cellular cholesterol which boosts MRR signaling. Molecular ablation and pharmacological repression of TSPO counteracted all this.

Conclusions

This work corroborates the NAM as a pathogenic conduit in the signalling of aggressive growth. In BCCs,

these newly uncovered contact sites underpin progression as well as resistance to ET which is primed by the impaired cholesterol metabolism. These data represent a substantial mechanistic gain which paves the way towards advanced personalized protocols for both diagnosis and treatment.

Alessandro Carrer

Fondazione Ricerca Biomedica Avanzata ONLUS, Padova

Metabolic-epigenetic connection in the malignant evolution of pancreatic neoplasia

Background and hypothesis

Pancreatic Ductal Adenocarcinoma (PDA) arises from precursor lesions, which are prevalent in healthy individuals. Despite carrying mutations at key driver genes, most lesions do not evolve into invasive cancer making neoplasia-to-adenoma transition a critical bottleneck in PDA evolution.

Fats- and fructose-rich diets increase PDA risk in humans and accelerate tumorigenesis in mice, but mechanisms are incompletely understood. We previously reported that PDA multi-step carcinogenesis is supported by deregulation of acetyl-CoA metabolism, which can be also impacted by diet. Mechanistically, acetyl-CoA availability dictates global levels of histone acetylation. We recently found that PDA progression is characterized by metabolic disturbances that promote the generation of nuclear acetyl-CoA from dietary lipids and fructose.

Building from the notion that epigenetic reprogramming is critical to release constraints of oncogenic transformation, we hypothesize that dietary inputs determine pro-tumorigenic licensing of chromatin at precursor cells through elevation of acetyl-CoA availability.

Aims

We seek to:

1) Dissect organelle-epigenome connection.

Nutrient cues are often sensed at subcellular compartments. We found that PDA cells rewire metabolic activities at mitochondrial, lysosomal and nuclear levels, but all converge at enhancing acetyl-CoA nuclear availability. We plan to characterize how compartmentalized metabolism integrate dietary inputs to reprogram histone acetylation.

2) Define diet-facilitated chromatin licensing in PDA progression.

Whether diet induces an adenoma-initiating epigenetic reprogramming is not known. We plan to profile chromatin accessibility in response to dietary inputs at single nucleus resolution, in both mouse and human pre-cancerous samples.

Experimental design

Proteomics of immunodeficiency-purified mitochondria revealed organelle-resident proteins that are deregulated upon malignant transformation of precancerous cells. Multiple high-resolution imaging techniques characterized impact of candidate proteins on mitochondrial morphology, while respirometry defined their functional role.

Expression of oncogenic KRAS (KrasG12D) in mouse pancreata leads to spontaneous development of neoplastic foci which infrequently become malignant lesions. These can be quantified by histological examination to evaluate the impact of metabolic interventions on autochthonous carcinogenesis.

Histone acetylation was probed using multiple assays, including immuno-staining for heavily-acetylated histone isoforms and making of chromatin accessibility (by single nucleus ATAC-seq).

Results

LFQ proteomics of purified mitochondria revealed several PDA over-represented proteins (vs pre-cancerous cells), involved in TCA cycle, electron transport chain (ETC), Coenzyme Q (CoQ) biosynthesis, FA oxidation, amino acids- and nucleotide metabolism (GO analysis, Reactome). Among the latter group is Nucleotide Di-Phospho Kinase D (NDPK-D, also known as NME4), a lesser characterized enzyme shown to dictate acetyl-CoA flux and lipid metabolism, and its binding partner RCC1-Like GTP Exchanging Factor (RCC1L, also known as WBSCR16) that together facilitate the reloading of GTP on dynamin-related proteins for the reshaping and movement of mitochondria. Immunohistochemistry (IHC) showed that NME4 expression increases in murine precursors lesions and tissue microarray in human tumors showed that high expression correlates with poorer histological grades. Functionally, NME4 silencing loosens mitochondrial cristae and reduces oxygen consumption, while prior evidence demonstrates that NME4/RCC1L augments OPA1 activity. NME4 silencing perturbs lipid homeostasis as assessed by BODIPY/OilRedO staining and MS lipidomics. These data indicate that mitochondria-resident nucleotide kinases are elevated during PDA progression to increase OPA1 function.

We next tested the hypothesis that OPA1 mediates oncogenic transformation. To this aim, we bred Opa1TG (mild Opa1 overexpression, from L. Scorrano) with KC mice to obtain KC;Opa1TG mice; these exhibited remarkably enlarged nuclei and elevated histone acetylation. Histological evaluation of 4-month-old animals showed the surprising presence of advanced carcinomas in 3/8 mice, while matched KC controls exhibited only low-grade PanINs as expected. Mechanistically, Opa1-overexpressing cells appear over-reliant on fatty acids (FA) oxidation and show enhanced histone acetylation.

Conclusions

Our data align with converging findings that show how NME4 and mitochondrial dynamics dictate preference for FA utilization. We conclude that NME4/OPA1-mediated mitochondria reprogramming influences acetyl-CoA metabolism and histone acetylation during PDA evolution.

Mauro Cives

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Integrating T cell redirecting strategies for the treatment of NETs

Background and hypothesis

Neuroendocrine tumors (NETs) overexpress somatostatin receptors (SSTRs). We hypothesize that T cell redirection against SSTRs may exert antitumor activity against NETs.

Aims

The primary aim of our project is to develop next-generation anti-SSTR chimeric antigen receptor (CAR) T cells capable to release a newly developed anti-SSTR bispecific T cell engager (BiTE) upon CAR engagement.

Experimental design

We have developed a second-generation, ligand-based, anti-SSTR CAR incorporating the somatostatin analog octreotide in its extracellular moiety, and demonstrated the antitumor activity of anti-SSTR CAR T cells both in vitro and in vivo (Mandriani et al, JITC 2022). More recently, in cooperation with Colleagues at Moffitt Cancer Center (FL, USA) we have designed a novel BiTE composed of 2 molecules of somatostatin-14 (SST14) linked with a single chain variable fragment (scFv)-based anti-CD3, and are currently testing its antitumor potential in vitro. In particular, after sequence optimization and subcloning into a vector (pAcGP67a) designed for protein expression in insect cells using Baculovirus, we have produced the recombinant protein in Trichoplusia-ni cells and performed isolation through nickel affinity chromatography. The recombinant protein was then characterized by SDS-PAGE and analytical size exclusion chromatography. The engager binding potential was evaluated by flow cytometry. Wild-type 293T cells (lacking SSTR expression) and 293T cells engineered to express SSTR2 have been then co-cultured with T cells in the presence of absence of the BiTE, and the release of IFN-g and Granzyme-B has been evaluated. The Incucyte technology has been used to evaluate the in vitro cytotoxic potential against SSTR- or SSTR+ 293T cells. We are currently assessing the in vitro antitumor activity of the anti-SSTR BiTE using autologous co-cultures of patient-derived NET tumoroids and patient-derived TILs as well as patient-derived NET tumoroids and circulating T cells enriched for neoantigen reactivities. The design of a CART_BiTE construct, possibly including a caspase-based safety switch, will follow.

Results

Anti-SSTR CAR T cells exerted antitumor activity against SSTR+NET cell lines in vitro. The killing activity was highly specific, as demonstrated by the lack of CAR T cell reactivity against NET cells engineered to express mutated variants of SSTR2/5 by CRISPR/Cas9. When adoptively transferred in NSG mice, anti-SSTR CAR T cells induced significant antitumor activity against human NET xenografts. Although anti-SSTR CAR T cells could recognize the murine SSTRs as shown by their killing ability against murine NET cells, no obvious deleterious effects on SSTR-expressing organs such as the brain or the pancreas were observed in mice. By flow cytometry, the BiTE was shown to bind CD3 on T cells as well as SSTRs on NET cells. The BiTE was able to specifically engage T cells, as demonstrated by the release of pro-inflammatory cytokines and the induction of anti-tumor killing activity.

Conclusions

T cell redirecting strategies directed against SSTRs have antitumor activity against NETs. Known peptide motifs may direct CAR T cell or BiTE targeting, providing a blueprint for therapeutic applications in a variety of cancers.

Gaia Codolo

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Tackling mechanisms of immune evasion in cancer: focus on the impaired antigen presentation.

Background and hypothesis

Colorectal cancer (CRC) ranks as the third most prevalent cancer and the second-leading causes of cancer related deaths. Its intricate pathogenesis involves dynamic interactions between developing cancer and surrounding tissue, including the immune system. A productive anti-tumor immune response hinges on effective antigen presentation via MHC-II by professional antigen-presenting cells (APCs) like macrophages, to CD4 T lymphocytes. Unfortunately, nearly two-thirds of CRC cases exhibit a lack of antigen-presenting molecules, correlating with diminished tumor-infiltrating T cells and an increased metastatic potential. Despite the abundant infiltration of tumor-associated macrophages (TAMs) in CRC, a heightened TAMs infiltration often correlates with poor prognosis.

While significant strides have been made in understanding the intricate relationship between the immune system and cancer, particularly in immunotherapy, CRC lags behind other malignancies in reaping the benefits of these advancements.

Our recent investigation uncovered a compelling link between macrophages and the immune receptor CD300e, activation of which resulted in impairment of antigen presentation by macrophages. The high expression of the receptor in CRC-infiltrating TAMs led to the hypothesis that CD300e could act as an immune checkpoint, modulating the expression of MHC-II molecules in CRC-infiltrating APCs.

Aims

The aims are devoted to define the molecular pathway(s) linking the activation of CD300e to the impaired exposure of antigen-MHC-II complexes in macrophages and to provide ground-breaking insight into mechanisms leading to the impairment of antigen presentation by APCs in tumors in a CRC model.

Experimental design

In vitro cell-based approaches have been adopted to define the pathway and the molecules leading to MHC-II decrease upon CD300e activation in monocytes/macrophages. The role of colorectal cancer cells and tumor extracellular matrix, and the involvement of CD300e, has been evaluated in vitro and ex-vivo on CRC samples. Finally, the impact of CD300e has been validated in a murine model of CRC in total KO and conditional KO mouse in the myeloid lineage.

Results

We discovered the existence of a pathway activated by CD300e, which acts as a negative regulator of STAT1 expression in monocytes. This leads to the downregulation of the MHC-II transactivator CIITA, resulting in a decrease in MHC-II expression. When primary human monocytes/macrophages were exposed to patient-derived tumor colon organoids or to decellularized extracellular matrices, they exhibited an anti-inflammatory response compared to those exposed to healthy matched specimens. Notably, in CRC bearing

KO mice a strong reduction of tumor growth has been observed compared to WT counterpart; tumors isolated from KO mice were characterized by the infiltration of macrophages displaying a pro-inflammatory profile, as well as a higher percentage of tumor-infiltrating CD4+ and CD8+ lymphocytes producing IFN-□ compared to WT.

Conclusions

In conclusion, our findings shed light on the role of CD300e in modulating the immune response in colorectal cancer. By elucidating the pathway through which CD300e influences MHC-II expression and the tumor microenvironment, we provide valuable insights into potential therapeutic targets for CRC. Targeting CD300e may offer promising avenues for immunotherapy in CRC, emphasizing the importance of further research into this pathway for the development of effective treatments.

Francesca Colombo

Consiglio Nazionale delle Ricerche, Segrate

Pharmacogenomics of opioid therapy for cancer pain

Background and hypothesis

Advanced cancer patients usually receive opioid therapy for pain. Unfortunately, 20-30% of patients do not benefit from the analgesic treatment and/or experience side effects such as nausea and vomiting. Pharmacogenetic studies have suggested that this interindividual variability is due to genetic variations in genes involved in opioid action or metabolism, but so far these data are not robust enough to support the development of clinical guidelines for personalized opioid therapy.

Aims

The main aim of our study is the identification of germline variants, at genome-wide level, associated with opioid efficacy and toxicity. Another aim of the study is to combine genome-wide genotyping and metabolic data to identify clinically relevant markers for the tailored opioid treatment of cancer patients.

Experimental design

We collected genomic DNA and/or blood samples from more than two thousand European advanced cancer patients treated with opioids (i.e., morphine, buprenorphine, fentanyl, and oxycodone). Personal and clinical information was collected, including data about the response to opioid treatment, both in terms of efficacy (pain intensity) and toxicity (nausea and vomiting), using standardized questionnaires and transformations to obtain numerical scores. Genomic DNA was genotyped using DNA microarrays. After quality controls and imputation steps, we performed genome-wide regression models between genotypes of >7 million variants and pain intensity (PI) and nausea-vomiting score (NVS). Functional role of the top-significant variants was investigated in silico. We are now genotyping additional 500 patients, to independently replicate the already obtained findings. In vitro studies will be carried out.

We will also perform a pilot pharmacometabolomic study using plasma samples of a subset of prospectively recruited patients, using mass spectrometry coupled with liquid chromatography. Using partial least squares-discriminant analysis, we will compare the metabolic profiles between opioid responders and non-responders and between patients with high and low NVS. Due to the limited number of patients with available plasma samples we will select metabolic pathways previously reported to be related to pain or opioids. We will test correlations between variants and metabolites, particularly those for which genetic control evidence is already available.

Results

Two genome-wide association studies (GWAS) were carried out to explore the association of variants with NVS and PI in 2,058 patients. In the first analysis, we found 68 variants associated with NVS (P-value < 1.0x10-6): 15 of them mapped on chromosome 2, in the NPAS2 gene, encoding a circadian transcription factor reported to be involved in opioid-induced alteration of sleep. Some of these variants were previously identified as splicing quantitative trait loci (QTLs) of the NPAS2 gene. The second GWAS identified five non-coding variants on chromosome 20 (P-value < 5.0x10-8), whose minor alleles were associated with a lower PI. These variants were 200kbp downstream of OPRL1, the Opioid Related Nociceptine Receptor 1 and were

reported as expression QTLs of this gene.

Conclusions

We performed the largest GWAS investigating the genetic bases of opioid response in advanced cancer patients, thus far. Our results emphasize the importance of performing large pharmacogenomic studies with the ultimate goals of improving personalization of cancer pain therapies.

Valerio Costa

Consiglio Nazionale delle Ricerche, Napoli

Targeting the (un)usual suspects in BRAF-mutated thyroid carcinomas

Background and hypothesis

Thyroid cancer is the most common endocrine-related cancer. Well-differentiated papillary carcinoma (PTC) has the highest frequency (80-85%), whereas anaplastic carcinoma (ATC) is the most aggressive form. Although they are mostly treated via surgery or radioactive iodine, these approaches often fail, especially at advanced stages. Despite the different prognosis, papillary and anaplastic tumors share BRAF mutations, accounting for 40-50% of cases. In contrast to the wild-type kinase, mutant B-raf is constitutively active and triggers downstream signaling even in absence of stimuli. In recent years, anti-cancer therapies based on B-raf inhibitors (BRAFi) have been effectively used in patients with BRAF-mutated tumors. Combinatorial treatments based on B-Raf and MEK inhibitors (MEKi) have higher efficacy compared to single drugs, and dabrafenib (DBR) plus trametinib (TRM) have been approved for advanced/metastatic BRAF-mutated ATC. However, acquired resistance limits drug efficacy, and the administration of higher doses - to restore drug sensitivity - leads to the selection of aggressive clones as well as to severe unwanted/side effects. Thus, investigating alternative options to overcome drug resistance in BRAF-mutated tumors is a clinical emergency.

Interestingly, tumors harboring BRAF mutations display a pronounced activation of MAPK-mediated signaling, paralleled by the induction of a unique set of coding and non-coding genes that makes these tumors targetable by "unconventional" drugs or molecules. Exploring the set of long non-coding RNAs induced downstream the BRAF mutation, we previosuly identified

COMETT as a natural antisense of MET oncogene, over-expressed in BRAF-like PTC. Considering its oncogene-like properties, we hypothesized that COMETT silencing in vivo may be used as new possible therapeutic option to treat BRAF-mutated PTC and ATC.

Aims

Our aims were:

- the characterization of the newly identified lncRNA COMETT and the evaluation of the phenotypic effects of its silencing in tumor cells in vitro;
- the definition of COMETT function through the identification of its protein interactors in tumor cells;
- in vivo validation of COMETT oncogenic capacity;
- the evaluation of COMETT targeting as a new possible therapeutic strategy for BRAF-mutated aggressive thyroid carcinomas

Experimental design

- A large set of COMETT-targeting ASO Gapmers has been extensively tested to achieve optimal silencing of different COMETT isoforms in BRAF-mutated PTC and ATC cell lines. CRISPR/dCas9 genome editing has been used to generate COMETT over-expressing cells.
- Multiple phenotypic and functional assays, together with transcriptome analysis, have been used to assess the oncogene-like properties of COMETT in vitro.

- COMETT-specific pull-down followed by mass spectrometry has been used to identify COMETT-interacting partners and ad hoc designed assays have been used to addrees its functional role in tumor cells;
- Cell-derived xenografts (CDX using aggressive ATC cell clones) assays by injection in NMRI nude mice have been used to evaluate in vivo the oncogenic capacity of COMETT, and to assess therapeutic efficacy of combining its silencing to currently used B-raf inhibitors.

Results

Using RNA pull-down coupled to MS we identified COMETT-interacting partners, with a significant enrichment of proteins involved in ribosome assembly, translation and splicing, further confirmed by COMETT-specific RIP followed by qPCR. SUnSET assay revealed impaired global translation rates, reduced levels of 80S ribosomal components and lower SR proteins' phosphorylation. Additionally, marked transcriptome changes were observed by RNA-Seq in COMETT-KD tumor cells, leading to the identification of a signature characterized by a marked repression of cell cycle genes and signaling by RTKs (especially EGFR) and TGFB family members, and the induction of the Unfolded Protein Response pathway, in line with COMETT binding to 80S riboproteins.

From a functional perspective, silencing of the COMETT long isoform had no effect on MET oncogene expression, even tohugh COMETT-KD tumor cells displayed strongly reduced oncogenic properties (e.g. viability, colony forming capacity and cell migration). Moreover, COMETT silencing in both PTC and ATC cell lines enhanced the anti-tumor effect of (sublethal) doses of BRAFi. Moreover, COMETT-KD cells treated with BRAFi at increasing doses were more sensitive to BRAFi effect than COMETT wild-type cells. In general, our results indicated that COMETT silencing - combined with BRAFi treatment - delayed MAPK re-activation (i.e. pErk rebound) in vitro.

COMETT targeting by ASO Gapmers in vivo has been evaluated using CDX (subq. injection of ATC cells) in NMRI nude mice, in collaboration with the PDX-Trace Facility in KU Leuven directed by our collaborator Prof. Eleonora Leucci. No significant tumor shrinkage was observed in mice treated with COMETT-specific ASO Gapmers, whereas a strong tumor growth reduction was measured in mice treated with BRAFi alone or in combination with COMETT-specific ASO, but no additive effects were observed. Currently, the oncogenic capacity of COMETT-KD tumor cells in vivo is under evaluation in CDX assays, where we expect to observe a reduced engraftment capacity for COMETT-KD cells.

Finally, keeping in mind the clinical need to identify alternative strategies to potentiate BRAFi-based therapy reducing the onset of drug resistance, we sought to test a - different from COMETT silencing, but complementary - strategy to target BRAF-mutated tumor cells. In this regard, we identified a signature of metabolic genes as substantially compromised in these tumors (vs normal cells) and we obtained very promising results by the simultaneous targeting of the glycolytic metabolism and B-raf.

Conclusions

In conclusion, our results indicate that COMETT lncRNA participates - at least partially - to 80S ribosome assembly and contributes to translation and, from a functional perspective, that its silencing has a strong impact on multiple oncogenic properties and tumor-associated signaling pathways. Moreover, COMETT-KD cells are more sensitive to BRAFi treatment and the re-activation of MAPK pathay, common upon BRAFi treatment, is delayed in vitro. Unfortunately, preliminary data show that its silencing is not as much effective on tumor growth in vivo and does not provide significant additive effects when combined with BRAFi.

However, during the last 2 years of the project, a possible complementary (or alternative) strategy to target BRAF-mutated thyroid carcinomas has emerged by our studies. Indeed, the simultaneous targeting of the

glycolytic metabolism and of B-raf kinase revealed to be promising, and it deserves further investigation.

Emilio Cusanelli

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Deciphering the functions of telomeric repeat-containing RNA TERRA in telomere length homeostasis of cancer cells

Background and hypothesis

Cancer cells attain unlimited proliferative capacity by maintaining telomere length homeostasis through activation of telomerase, which occurs in 90% of human cancers, or engaging in alternative telomere lengthening mechanisms, known as ALT, relying on homologous recombination. These processes are essential to tumorigenesis and represent attractive therapeutic targets. The long noncoding RNA TERRA is transcribed from telomeres and plays key roles in telomere biology. TERRA molecules interact with telomerase in human cancer cells. Furthermore, TERRA transcription deregulation impairs telomere function in ALT cancer cells. These findings indicate that TERRA is implicated in telomere length homeostasis of cancer cells. Nevertheless, the function of TERRA in the regulation of human telomerase remains to be defined. Moreover, whether TERRA acts as a trigger of ALT has not been investigated.

We hypothesize that TERRA transcripts regulate telomerase activity and contribute to ALT activation. Thus, TERRA represents a critical regulator of telomere length homeostasis in cancer cells.

Aims

We aim to characterize the function of TERRA in the regulation of telomerase in cancer cells and define the involvement of TERRA in ALT activation using both cancer cell lines and multicellular organisms.

Experimental design

We use single molecule inexpensive RNA FISH (smiFISH) combined with immunofluorescence (smiFISH/IF) to simultaneously visualize TERRA, telomerase RNA and telomeres in single cells, as well as RT-qPCR and northern blot to quantify TERRA expression during telomere elongation by telomerase in cancer cell lines, and during ALT activation in cancer cells and multicellular model organisms.

We employ antisense oligonucleotides to deplete TERRA molecules in cells and study the consequences on telomerase function and dynamics, both in fixed and living cells, and the impact on ALT activation using cancer cell lines and a zebrafish ALT brain cancer model.

Results

Our results indicate that telomeric TERRA transcripts impair telomerase access to chromosome ends. We found that TERRA colocalizes with telomerase RNA hTR both in the nucleoplasm and at telomeres. Yet, the telomeric fraction of TERRA and TERRA-hTR colocalizing molecules markedly decreases during telomere elongation, while the number of telomeres with telomerase molecules devoid of TERRA rises. Interestingly, ALT induction resulted in the opposite scenario, with TERRA transcripts relocating to telomeres at early stages of ALT. Consistently, using C. elegans, the only multicellular organism able to survive telomerase genetic inactivation by developing ALT, we observed that ALT prone genetic backgrounds express high levels

of TERRA before ALT induction.
TERRA depletion by antisense oligonucleotides was found to promote telomerase RNA hTR localization to telomeres, increasing its residence time and half-life at chromosome ends, as estimated by live-cell imaging approaches. Preliminary results indicate that TERRA depletion in a zebrafish ALT brain cancer model results in amelioration of the phenotype linked to tumor development and increased survival of ASO-injected tumor bearing fishes.

Conclusions

Our findings indicate that telomeric TERRA transcripts inhibit telomere elongation by telomerase, impairing telomerase access to chromosome ends. Furthermore, TERRA may be involved in the early stages of ALT, contributing to ALT activation during tumorigenesis. Thus, TERRA transcripts are important players in telomere length homeostasis of cancer cells.

Raffaella Di Micco

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Expoiting senescence immunogenicity for leukemia treatment

Background and hypothesis

Cellular senescence, characterized by irreversible growth arrest induced by various factors, plays a significant role in cancer, including acute myeloid leukemia (AML). Despite its potential tumour suppressive functions, the molecular mechanisms underlying the crosstalk between senescent cancer cells and the immune system remain unclear. AML remains challenging to treat, with high-dose chemotherapy being the current standard of care, often leading to treatment failure due to chemo-resistant tumours. We hypothesize that senescence is a key mechanism driving leukemia immunogenicity. As genetic aberrations alone do not fully explain senescence competence in AML patients, we propose that changes in the epigenome may dictate the interaction between senescent cells and the adaptive immune system. Furthermore, we anticipate that combining senescence-inducing or epigenetic therapies with immune checkpoint blockers (ICBs) will potentiate therapeutic outcomes. This study aims to elucidate the role of senescence in AML immunogenicity and explore potential therapeutic strategies for enhancing treatment efficacy.

Aims

1) Develop models to assess therapy induced senescence in primary AML
2) Exploiting the role of epigenetics in TIS immunogenicity
3) Dissect the interplay between senescent cells and the adaptive immune system

Experimental design

We exploited ex-vivo cultures of primary AML samples or cell lines undergoing senescence upon different treatments. Multiparametric flow cytometry analysis integrated with quantitative imaging will be used to measure senescent markers and human leukocyte antigen (HLA) expression in a cohort of primary AML samples at diagnosis and upon ex-vivo therapy. The mixed lymphocyte reaction assay will be performed to evaluate T cell activation in response to senescent blasts. ATAC-seq will be exploited for assessing histone modifications associated with transcriptional activation or repression.

Results

By combining transcriptional and cell-based evaluation of senescence markers in primary AML patient samples, we identified two groups of patients based on their ability to accumulate SA-β-GAL upon ex vivo chemotherapy and defined them as Senescence High or Senescence Low samples. Consistently, only in Senescence High patients, we found upregulation of HLA class I and II molecules and their regulators, both at RNA-seq and protein level, revealing a link between TIS establishment and increased blasts immunogenicity. Consistently, senescent AML samples activated autologous CD4+ and CD8+ T cells, leading to improved recognition of leukemic blasts ex-vivo as well as in in vivo patient derived xenografts models. Moreover, we found enhanced T cell activation for both T cell subsets when combining TIS and immune checkpoint blockade (ICB) therapy, suggesting that senescence competence upon chemotherapy may be exploited to stratify AML patients that would benefit from this treatment. Lastly, driven by the hyphothesis

that senescence competency of patients and the consequent increased immunogenicity may be driven by epigenetic modalities, we identified an epigenetic drug, impacting on the polycomb repressor complex 2 (PRC2) catalytic subunit EZH2, able to revert the immunological mechanisms at the basis of senescence low patients, leading to the reactivation of immune cells against the tumour.

Conclusions

Overall, our findings uncover a novel link between senescence induction and leukemia immune recognition by T cells via upregulated components of the antigen presentation machinery, elucidating the basis for conceptually novel senescence-based targeted immunotherapeutic regimens for AML patients.

Alessandro Fantin

Università degli Studi di Milano, Milano

Endothelium heterogeneity in the control of organotypic vascular morphogenesis in health and cancer

Background and hypothesis

New blood vessel growth, or angiogenesis, is a hallmark of all solid cancers, whereby tumours, in the absence of blood supply, are not able to grow. Therefore, an effective treatment for solid tumours would be to interfere with the main angiogenic signalling process to starve the tumour. However, anti-angiogenic therapies had limited success prompting the need for better strategies. Possible avenues for escape from antiangiogenic therapies may lie in the stromal components of the tumour microenvironment, such as tumour-associated macrophages (TAMs) that, in most cases, promote the progression and malignancy of tumours, as well as the angiogenic switch required for these processes. I previously unveiled that tissue-resident macrophages interact with nascent blood vessel sprouts and act as cellular chaperones to promote vessel anastomosis during vascular morphogenesis. However, the molecular mechanisms involved in macrophage-promoted vessel anastomosis are still mostly unclear.

Aims

Reveal novel molecular and cellular mechanisms by which TAMs promote cancer angiogenesis to provide new therapeutic targets for cancer therapy.

Experimental design

We combined bulk and single cell transcriptomics to functional studies as well as biased and unbiased approaches to identify the signalling pathways that are involved in macrophage-blood vessel interactions during vascular morphogenesis in health and cancer.

Results

The tyrosine kinase receptor KIT appeared dispensable for physiological angiogenesis, but provided angiogenic advantage in endothelial cells in which it was expressed. Since KIT was enriched in lung endothelial cells with progenitor-like properties, it might play an important role in vascular expansion in pathological conditions, such as lung carcinoma growth. Endothelium-driven activation of the chemokine receptor CXCR4 prevented vascular overgrowth by regulating notch signalling specifically in the brain and not in other organs. We also found that the brain endothelium was intrinsically more sensitive compared to endothelial cells of other organs to the local source of IGF1 provided by macrophages, prompting us to assess the relevance of this pathway for glioblastoma expansion. To improve representation of the functional endothelial cell transcriptome, we combined single cell RNA-seq with the generation of the BulkECexplorer, a new transcriptomic tool to interrogate gene expression in different organ endothelial cells and predict active versus leaky genes. Finally, we found opposing effects on breast cancer growth induced by macrophage depletion at different temporal windows from tumour onset, with early depletion associated with faster tumour growth, whereas late-stage depletion significantly reduced tumour growth and vascular remodelling.

Conclusions

We found organ-specific molecular pathways that differentially regulate the expansion of the blood vasculature both involving or not involving a crosstalk with macrophages. These findings have important relevance for devising anti-tumour angiogenesis strategies that take into account the different molecular signatures of the organ microenvironment in which each tumour develops.

Luca Fava

Università degli Studi di Trento, Trento

Dissecting p53-dependent ploidy control through a whole genome loss of function screen

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Background and hypothesis

The p53 tumor suppressor is well-established in restraining the proliferation of polyploid cells. Tumors with Whole Genome Doubling (WGD) exhibit TP53 mutations at a much higher prevalence. Our work has highlighted the importance of the PIDDosome, a multiprotein complex devoted to activating Caspase-2, in the p53 response to acute WGD. Caspase-2-dependent cleavage of MDM2 is necessary and sufficient for p53 activation upon acute WGD. We hypothesized that cells don't measure cellular ploidy but utilize centrosome numbers as a proxy to detect WGD events.

Aims

Our aim was to develop a fluorescent reporter for PIDDosome activation to study centrosome-dependent signaling at the single-cell level. Using this tool, we aimed to identify factors influencing the ability of cells to mount a p53 response during acute WGD.

Experimental design

We employed highly multiplexed perturbations with a genome-wide CRISPR library. Cells expressing the library were forced to undergo WGD before flow cytometric separation to isolate fluorescent vs. non-fluorescent cells. Next-generation sequencing allowed assessing the sgRNA distribution across conditions.

Results

Genetic ablation of centrosomal proteins impaired the ability of cells to sense acute WGD via the PIDDosome. Perturbation of a specific centrosomal substructure hindered MDM2 cleavage, leading to an abrogated p53 response. Additionally, disrupting the p53 binding to the MDM2 promoter attenuated MDM2 proteolysis. A consistent group of hits from our screen were identified as essential for p53 target gene transactivation.

Conclusions

Our findings strongly support that amplified centrosomes are crucial for cells to sense acute genome doubling. Furthermore, centrosomes can locally concentrate essential signaling proteins, directly influencing cell fate.

Gennaro Gambardella

Fondazione Telethon, Pozzuoli

Single cell lineage tracing reveals clonal dynamics of anti-EGFR therapy resistance in triple negative breast cancer.

Background and hypothesis

Triple-negative breast cancer (TNBC) is a highly heterogeneous and aggressive breast cancer subtype characterized by metastatic progression, poor prognosis, and the absence of targetable biomarkers. Neoadjuvant chemotherapy is initially highly effective on TNBCs but about 30%–50% of the patients rapidly develop resistance associated with higher mortality. Despite Epidermal Growth Factor Receptor (EGFR)-activating mutations and amplifications (≥ 5 copies) are uncommon in TNBC, the majority of primary TNBCs exhibit enhanced expression of EGFR because of an increase in gene copy number (three to four copies), thus representing a valuable vulnerability for TNBC patients. However, unlike other tumour types where inhibition of wild-type EGFR by monoclonal antibodies or tyrosine kinase inhibitors (TKIs) is beneficial, EGFR-targeted therapies in TNBCs have shown variable and unpredictable clinical responses (1.7% to 38.7%). Indeed, we and others found no significant correlation between EGFR status (i.e. copy number, mRNA, protein, or phospho-protein levels) and response to anti-EGFR therapies. Moreover, genomic variants that were found to predict anti-EGFR resistance in other tumour types are infrequent in TNBC patients, suggesting that non-genomic resistance mechanisms must be at play in TNBCs. As a result, the lack of predictive biomarkers of response to anti-EGFR therapies in TNBC has hampered the translation of EGFR inhibitors in breast cancer.

Single-cell RNA sequencing technologies have recently emerged as powerful tools to study intra-tumour heterogeneity and to reveal key genes that change in response to an external stimulus such as drug treatment. However, when monitoring the emergence of drug-resistant cell populations, it is crucial to identify and compare how cancer cells' surviving lineages (i.e., clones) adapt to the treatment. Recently, bulk RNA sequencing and cellular barcoding, a technique that uses distinct DNA sequences to mark each cell, have provided the possibility of following cancer progression, metastasis dissemination, and cell differentiation at a single clone level. Hence, developing novel methods that couple DNA marking techniques with techniques measuring cellular states at single-cell resolution is essential to determine the different adaptive trajectories leading to drug resistance. Once these two techniques are coupled, prospective lineage tracing analyses can be used to study single clone dynamics during treatment to identify mechanisms driving drug adaptation. In contrast, retrospective analyses can be used to trace back in time and reconstruct pre-existing mechanisms of drug resistance by comparing the transcriptional states of tolerant and sensitive clones before the treatment.

Aims

(1) Development of a platform to probe cell state transitions of TNBC cells in response to drug treatment.

(2) Elucidation of the key genes that mediate the transition between the sensitive and resistant state of cancer cells in response to drug treatments.

(3) In-vitro validation of biomarker genes of drug resistance.

Experimental design

We integrate methods for cellular barcoding and single-cell transcriptomics to enable cell lineage tracing and explore the subclonal evolution of adaptation in an established preclinical model of TNBC in response to incremental concentrations of afatinib, a potent TKI that irreversibly inhibits both EGFR and HER2 receptors.

Results

Retrospective lineage tracing data analysis uncovered a pre-existing subpopulation of rare afatinib-tolerant cells displaying distinct biological features, such as elevated mRNA levels of IGFBP2 (Insulin-Like Growth Factor Binding Protein 2). We demonstrate by chemical and genetic manipulations that IGFBP2 overexpression is sufficient to render TNBC cells tolerant to afatinib treatment by activating the compensatory IGF1-R signalling pathway. On the other hand, prospective lineage tracing highlighted additional adaptive mechanisms, including lysosome biogenesis, reactive oxygen species (ROS) homeostasis, and fatty acid metabolism. Finally, by linking reconstructed mechanisms of drug resistance with deep learning techniques, we developed an algorithm to computationally predict afatinib response based on the transcriptional status of TNBC cells.

Conclusions

Our findings provide a new understanding of the intricate signaling network underlying EGFR-targeted therapy resistance in TNBC, which can help devise novel strategies for TNBC patient stratification and therapeutic intervention.

Fabio Giavazzi

Università degli Studi di Milano, Milano

Optical fingerprinting of metastatic potential

Background and hypothesis

Clinical diagnoses and decisions are routinely based on visual inspection of medical images and their consequent use for the classification of diseases. One of the main factors limiting the impact of automated image analysis for diagnostic and patient monitoring purposes is the lack of suitable imaging markers bearing a direct and quantitative relation with the physiological, biological, or physical properties altered by the disease. This lack is particularly significant for cancer, a disease for which advances in this direction may lead to immediate results of clinical relevance. This project builds on the emerging observation that physical forces, and their interplay with biochemical signaling, play a key role in determining the behavior of cell collectives. In particular, the geometrical and topological properties of the cells composing a tissue and their spatial arrangement (the tissue structure, in physical terms) strongly correlate with their motility. Since the ability to overcome kinetic arrest is a condition for cancer cells to disseminate, we posit that the quantitative assessment of the structural properties of tissues is a powerful and uncharted way to assess the invasive and metastatic potential of cells quantitatively.

Aims

This project aims to identify quantitative, biophysically motivated imaging markers, that can be automatically extracted from microscopy images or image sequences of in vitro, ex vivo, or histopathological samples. These biomarkers provide a synthetic Optical Fingerprint (OFP) capturing the structural properties of the tissue, which could be used to assess its dynamical state and to predict the invasive and metastatic potential of cancer cells, with a specific focus on breast carcinoma.

Experimental design

A panel of quantitative imaging markers, obtained with a portfolio of complementary image analysis tools, leading to the OFP is defined. The reliability, robustness, and predictive power of the OFP is improved and confirmed by performing in vitro experiments on model systems of increasing complexity. The second part of this project is devoted to obtaining a proof of principle of the validity of the proposed approach in a clinical setting, to predict metastatic propensity from the analysis of histopathological images.

Results

By considering a variety of two- and three-dimensional models, we show that cellular unjamming, a phase transition mediated by alterations in tissue-level mechanical properties and collective motility, considered a key process promoting cancer invasion, has specific dynamic, morphological and structural signatures that can be effectively captured by image analysis. In agreement with previous reports, we identify the increased asymmetry in cell and nuclear shape, as captured by the aspect ratio (AR), as a simple yet robust marker of unjamming. Preliminary results obtained from a cohort of pure ductal carcinoma in situ (DCIS), DCIS with local areas of infiltration, or overtly invasive ductal carcinoma (IDC) show that pure, indolent DCIS display reduced nuclear AR, indicative of a more jammed, solid-like state, whereas the AR progressively increased in locally infiltrative DCIS and invasive IDC, consistent with the notion that aggressiveness of the disease

correlates with enhanced tissue fluidity.

Conclusions

The proposed model-based biophysical approach represents a valuable route to obtain quantitative information with direct biological implications from microscopy images, particularly when time-resolved data are available. With the view of the systematic application to histopathological samples, the integration within the existing image analysis platform of available machine-learning-based tools, both in the feature extraction and classification phases, could drastically improve current performance.

Luca Lanzanò

Università degli Studi di Catania, Catania

Optical nanoscopy to investigate the origin and evolution of oncogene-induced genomic damage

Background and hypothesis

Activation of oncogenes may trigger the generation of genomic damage through alterations of the dynamics of DNA replication, transcription and DNA damage response (DDR). However, despite their potential importance in cancer development, the molecular mechanisms that underlie oncogene-induced genomic damage are still poorly understood.

Our core hypothesis is that oncogene activation induces an alteration in the spatio-temporal organization of DNA replication and/or transcription, favoring the occurrence of replication and transcription stress at specific sites in the genome.

Aims

The main goal of the proposal is to elucidate, by means of advanced optical microscopy (including confocal and super-resolution imaging), molecular details of the origin and evolution of oncogene-induced genomic damage. Specifically, I aim to visualize if the spatio-temporal organizations of transcription and replication sites are altered following oncogene activation and if these alterations correspond to an increase of genomic damage. In addition, I aim to visualize the role of alterations of the DDR on the evolution of oncogene-induced genomic damage.

Experimental design

We apply advanced confocal and super-resolution imaging in cellular models of oncogene activation, to visualize alterations of transcription, replication and DDR (and the resulting DNA damage) directly in the intact nuclear space. Specifically, we exploit the U937-PR9 cell line, an in vitro model of acute promyelocytic leukaemia, to shed light on the molecular basis of DNA damage generated by PML-RARα oncogene activation. In order to analyze a high number of cells, high resolution microscopy is integrated with a high-throughput computational platform for cell-cycle multi-parameter analysis.

Results

We search for alterations in the spatial distributions of replication and/or transcription, following oncogene activation. We find that activation of the oncogene induces a significant increase in the fraction of transcription sites colocalized with PML/PMLRARa, due to disruption of physiological PML bodies into a large number of PML-RARa microspeckles. We also characterize DNA damage foci amount, size, and location relative to the PML-RARα oncoprotein expression, as well as colocalization fraction, at the single-cell level. We discuss distinctive markers of the DNA damage triggered by the oncogene, which can relate to the effects of the PML-RARα protein.

Conclusions

Conclusions

High resolution imaging enables observation of the effects of oncogene activation in singe cells. The results

suggest that PML-RARα activation may induce alterations on chromatin organization and specifically in the coordination of replication and transcription.

Luigi Leanza

Università degli Studi di Padova, Padova

Impaired mitochondrial metabolism and ER-mitochondria contact sites affect signaling in cancer cells

Background and hypothesis

Mitochondria are organelles not only involved in cellular respiration but also in several other pathways important for cell life and death. They are not isolated within the cells but are closely interconnected with other organelles, among which the Endoplasmic Reticulum (ER). Defective ER-mitochondria crosstalk and ER stress impacts on several cellular functions as well as on important intracellular pathways that promote the cancer development. The dynamic regulation of ER-mitochondria contact sites affects mitochondrial physiology and adaptation of cellular metabolism to nutrient availability. Modulation of ER-mitochondria contacts length and the distance between the two organelles impacts both on their function and on cellular signaling.

Aims

Modulation of ER-mitochondria contacts have a role in cancer development and resistance to pharmacological therapy by impacting on cellular bioenergetics and metabolism. We aimed to elucidate how ER-mitochondria contact sites and cellular metabolism modulation could impact on signaling and tumor progression.

Experimental design

We propose: 1) to confirm in vitro and in vivo that ER-mitochondria contact sites can affect signaling pathways using genetic modulation of proteins involved in contacts formation; 2) to assess in vivo whether cellular metabolism and nutrients affects signaling and tumor development/progression depending on mitochondrial function and on contacts formation; 3) to assess whether tumor development and/or the response of the tumors to chemotherapy is affected differently depending on diet.

Results

We described for the first time the "mitochondria-Wnt axis". In detail, pharmacological or genetical impairment of the respiratory chain complexes causes a reduction in the ATP synthesis, leading to a decrease in the ATP present in the microdomains between the ER and the mitochondria. The reduced ATP induces a diminution of the import of calcium inside the ER triggering ER-stress, which in turn downregulate Wnt signaling and finally impact on cancer cell proliferation.

In addition studying the role of Transglutaminase type 2 (TG2) maintenance of ER-mitochondria contact sites in cancer, we identify a new role of TG2 in modulating Wnt signaling, by stabilizing beta-catenin and supporting its nuclear translocation. This TG2's scaffold activity to support nuclear translocation has been shown to act in regulating "phenotype switching" of melanoma cells. In particular, TG2 can directly bind MITF, an important transcription factor involved in melanoma cell differentiation, and support its nuclear translocation. In absence of TG2, MITF cannot enter the nucleus and does not mediate melanoma cell differentiation, leaving them undifferentiated and more prone to form larger metastasis, as shown by in vivo

experiment with melanoma mouse models. Importantly, these effects impact also on the capability of melanoma cells to recruit immune cells.

Finally, we showed that a reduction of ER-mitochondria contacts, by downregulation of tethers or metabolism rewiring, can tune cancer cells intracellular signaling both in vitro and in vivo, ultimately impacting on cancer development and cancer cells sensitivity to drugs.

Conclusions

These findings reveal that affecting ER-mitochondria tethering may be beneficial against cancer by altering the cellular signaling, and in turn sensitizing tumor cells to chemotherapeutic treatment.

Laura Maggi

Università degli Studi di Firenze, Firenze

Effects of adenosine A2A-receptor inhibitors on effector function of human T lymphocyte subsets

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Background and hypothesis

Cancer immunotherapy represent a novel, high potential strategy for oncological treatment. Adenosine is an endogenous purine nucleoside and its immunosuppressive role in the tumor microenvironment made the Adenosine receptor A2A AR-mediated pathway a possible target for anti-tumoral therapy.

Aims

The aim of this work was to evaluate the efficacy of new synthetic A2A AR antagonists based on a thiazolo[5,4-d]pyrimidine nucleus and characterized by an high affinity for the A2A AR.

Experimental design

These 5 new developed synthetic A2A AR antagonists supplied by the Chemistry laboratory of the University of Florence, named from A1 to A5, as well as the compound ZM 241385, already known and tested in the literature, were tested on proliferation and cytokine production of CD4+ and CD8+ T lymphocytes and on the different T helper subsets (Th1, Th2, Th17), stimulated in presence of Adenosine or its synthetic analogue CGS 21680.

Results

We found that the Adenosine agonist CGS 21680 causes an inhibition of proliferation and cytokine production on CD4+/CD8+ T cells comparable to that observed with the use of the physiological Adenosine. Moreover, we found that ZM 241385 and some of other synthetic compounds (A1-A5) restore the normal proliferative rate of cells and cytokine production, even if with different potency.

Conclusions

These results suggest that this class of drugs can constitute an innovative therapy in the field of anticancer immunotherapy. Future research should include the evaluation of the A2AR-Adenosine axis' effects on both tumor cells and T regulatory cells as these drugs may also be able to reduce both the proliferation of tumor cells and T reg-mediated immunosuppression.

Gennaro Napolitano

Fondazione Telethon, Pozzuoli

Selective modulation of mTORC1 signaling in B cell lymphoma

Background and hypothesis

The mechanistic target of rapamycin complex 1 (mTORC1) is a nutrient- and stress-sensing protein kinase that plays a central role in the control of cell metabolism and growth. The activation of mTORC1 requires its amino acid-dependent recruitment to the lysosomal membrane via the Rag GTPases, obligate heterodimers of RagA or B bound to RagC or D, and its growth factor-dependent activation by Rheb. Different nutritional inputs impinge on mTORC1 activation status to induce the phosphorylation of its multiple substrates, such as S6K and 4E-BP1, which are involved in the regulation of anabolic processes, as well as the transcription factors TFEB and TFE3, which are master transcriptional modulators of cell catabolism. However, whether and how mTORC1 can differentially phosphorylate its substrates in response to specific stimuli has long remained elusive.

Deregulation of mTORC1 signaling is commonly observed in a wide variety of solid and hematologic cancers. Accordingly, numerous mTORC1 inhibitors have entered clinical trials for cancer treatment. Interestingly, mutations affecting several mTORC1 signaling components, such as the v-ATPase complex, are frequently found in patients with follicular lymphoma (FL), one of the most common forms of non-Hodgkin lymphomas (NHL). However, the mechanisms by which these mutations affect B cell functions and contribute to lymphomagenesis are still poorly understood.

Aims

A major goal of our project is to define the mechanisms by which mTORC1 controls the phosphorylation of its downstream targets, which is relevant for the modulation of cell metabolism both in physiological contexts and cancer. Based on our preliminary data, we hypothesized that the Rag GTPases act as a molecular switch to selectively control the modulation of mTORC1-mediated responses. Furthermore, we aimed at characterizing the role of the v-ATPase complex in this process, by defining the mechanism by which FL-associated v-ATPase mutations affect mTORC1 signaling and lymphomagenesis.

Experimental design

We use multiple approaches, ranging from cell biology, biochemistry, structural biology, and in vivo studies, to determine the role of the Rag GTPases and of the v-ATPase complex in the modulation of mTORC1 signaling and the relevance of this pathway in follicular lymphoma.

Results

We discovered a substrate-specific mTORC1 pathway, specifically controlled by the RagC/D GTPases, that regulates the function of TFEB and other MiT-TFE factors. Owing to a “non-canonical”, RagC/D-mediated substrate recruitment mechanism, the phosphorylation of TFEB by mTORC1 is highly sensitive to the activation status of RagC/D but insensitive to growth factor-mediated activation of Rheb. By contrast, the “canonical” mTORC1 substrates S6K and 4E-BP1 behave in the opposite manner. This mechanism enables mTORC1 to promote selective metabolic responses to diverse environmental cues via differential substrate

phosphorylation. Furthermore, we found that the v-ATPase complex plays a key role in the regulation of TFEB phosphorylation by mTORC1, via specific modulation of RagC/D GTPases. Importantly, the constitutive activation of TFEB, due to de-regulation of RagC/D activity, is a key feature of cells carrying FL-associated v-ATPase mutations and plays a major role in promoting cell proliferation and survival.

Conclusions

Our data uncover a novel substrate-specific mechanism, which we refer to as “non-canonical” mTORC1 signaling, that enables differential modulation of mTORC1-mediated responses. I will discuss the mechanisms regulating mTORC1 substrate-specific activity and the relevance of these processes in B cell lymphoma.

Chiara Naro

Università Cattolica del Sacro Cuore, Roma

Alternative splicing dysregulation as a novel therapeutic vulnerability for triple-negative breast cancer

Background and hypothesis

RNA splicing dysregulation has recently emerged as a featuring trait and therapeutic vulnerability of triple-negative breast cancer (TNBC), one of the most aggressive breast cancer subtypes, which is still lacking of efficacious targeted therapies. NEK2 is a splicing factor kinase that we found significantly upregulated in TNBC compared to other breast cancer subtypes. Furthermore, by transcriptomic analysis we found that NEK2 ablation in TNBC cells exerts a genome-wide regulation on gene expression and alternative splicing. It is thus conceivable that NEK2 might contribute to the oncogenic dysregulation of transcriptional and post-transcriptional programs in TNBC.

Aims

We aimed to analyze the functional relevance of NEK2-regulated transcriptional and post-transcriptional program for TNBC biology and to study the mechanisms underlying such regulatory activity, in order to design novel therapeutic strategies to interfere with this oncogenic kinase.

Experimental design

By functional annotation analysis of genes in the NEK2-sensitive splicing signature we investigated the biological processes affected by the splicing regulatory activity of this kinase in TNBC cells and by query of transcriptomic data from primary TNBC we evaluated their prognostic value. Putative effectors of NEK2-mediated post-transcriptional changes have been searched by bioinformatics analysis of regulatory motifs enriched in NEK2 target exons and by proteomic analyses of NEK2 nuclear interactome. Guided by these analyses, we tested the functional impact of modulating the expression of selected NEK2-regulated splice-variants on TNBC cells oncogenic features.

Results

We found that NEK2 promotes a pro-mesenchymal splicing program in TNBC, including splice-variants discriminating TNBC from other breast cancer subtypes and correlating with poor prognosis. Both NEK2 and its functionally interacting RNA-binding protein SAM68 enhance the invasive and migratory properties of TNBC cells. Remarkably, depletion of select NEK2-sensitive splice-variants that are prognostic in TNBC patients is sufficient to interfere with TNBC cell morphology and motility, suggesting that NEK2-mediated dysregulation of alternative splicing is relevant for TNBC aggressive phenotype.

Conclusions

Our study uncovers an extensive splicing program modulated by NEK2 involving splice variants that confer an invasive phenotype to TNBCs and that might represent, together with NEK2 itself, valuable therapeutic targets for this disease.

Elisabetta Palazzo

Università degli Studi di Modena e Reggio Emilia, Modena

Neurotrophin network as a novel target for the treatment of squamous cell carcinoma

Background and hypothesis

Cutaneous squamous cell carcinoma (cSCC) is the second most prevalent form of skin cancer, and its incidence is constantly increasing worldwide. Several evidence display a heterogeneous expression of the neurotrophin network in head and neck cancers, but only a few reports described the expression and function of neurotrophins (NTs) and their receptors (NTR; CD271 and Trk receptors) in cSCC.

Aims

Given the importance of NTR in epidermal homeostasis, this work aimed to study the involvement of NTs and NTR in the pathomechanisms of cSCC, with the final goal of identifying new therapeutic targets.

Experimental design

cSCC patient-derived spheroids have been generated, reflecting the behavior of cSCC and its subpopulations, the stem-like Rapidly Adhering cells (RAD), and the more differentiated Not-RAD cells (NRAD). NT and NTR expression and function were assessed by 3D assays and molecular analysis in vitro. Patient-derived cell metastatic capacity was observed by transgenic zebrafish avatar models. By conditional deletion of CD271 in the murine epidermis (cKO), the total skin and keratinocyte profile were clarified by imaging, protein, and RNA analysis. Susceptibility to inflammation and carcinogenesis protocol were also applied.

Results

Our data revealed that CD271 level is higher in Well-Differentiated cSCC spheroids, while Moderately-to-Poorly Differentiated cSCC spheroids mostly express TrkA, showing a different NTR expression during cSCC clinical stages. At the cellular level, TrkA was highly expressed and localized on the RAD cell membrane, while CD271 was found at both membrane and within cell nuclei in NRAD cells, suggesting potential regulatory mechanisms. CD271-overexpression reduced malignancy features, with a major action on the stem-like RAD spheroids. Conversely, Trk-silencing promotes a reduction of cell growth, as well as a decrease in mitogenic signaling. By zebrafish models, we proved that RAD cell injection produced a higher number of metastasis than NRAD. While CD271 activation decreases metastasis, Trk receptor inhibition favors a major percentage of in-place conditions, thus corroborating in vitro results. Moreover, immune cell ablation induced a significant increase of cSCC metastases and leucocyte recruitment in zebrafish, which correlates with tumor-killing according to CD271 or Trk expression modulation. These data were supported by the novel conditional CD271 KO mouse models (full or TAM-inducible), which recapitulated the role of CD271, and the NT network, in the skin. cKO skin shows profound epidermis disorganization, higher proliferation rates, and a decrease of differentiation. Immune cell infiltration was also highlighted at different stages of skin development. Whole transcriptome analysis identifies differentially expressed gene enrichment in PI3K-AKT-mTOR, MAPK, and Integrin-mediated cell adhesion signaling pathways. Specifically, deregulation of PI3K/AKT

and PKCα/ERK expression has been found. Moreover, CD271 deletion promotes increased proliferation and an "activated profile" of the epidermal cells, which means having a higher chance of developing malignant skin features, as demonstrated by a greater response to inflammatory stimuli and a major incidence of skin lesions.

Conclusions

Our results identify specific NTR-associated signaling in cSCC, which brings on immune cell potential activation towards tumor cell death. In detail, NTR, specifically CD271, contribute to the modulation of active cancer-related paths and could be implied in novel therapeutic strategies.

John Charles Rotondo

Università degli Studi di Ferrara, Ferrara

High prevalence of Merkel cell carcinoma in autoimmune disease affected patients treated with biological drugs

Background and hypothesis

Merkel cell carcinoma (MCC) is a rare and aggressive skin cancer. Two MCC subtypes have been identified, i.e., Merkel cell polyomavirus (MCPyV)-positive and MCPyV-negative subsets. MCC molecular characteristics have been partially uncovered. MCPyV is a small DNA tumor virus which exerts its oncogenic activity by large/small T (LT/sT) viral oncoproteins. MCPyV, ubiquitous in humans, establishes an early-in-life, lifelong and asymptomatic infection, while conditions of immune impairment lead to a viral replication and an increased oncogenicity. A high MCC rate has been documented in immunosuppressed patients under treatment for autoimmune diseases (ADs). The impaired host antiviral immune response is an MCC-risk factor when biologic drugs are used in the AD patient (ADP) management. Therefore, longitudinal investigations on MCPyV serology in ADPs under immunosuppressive therapy, could delineate the identification of MCC-risk patients.

Aims

Aim I. To analyse the MCPyV serology in ADPs under immunosuppressive therapy with biologic drugs during a long-term follow-up. Aim II. To investigate the molecular characteristics of MCC tissues through histological tumor evaluation and investigation of MCPyV molecular features.

Experimental design

This 5-year project provided the enrolment of 650 biologics-treated ADPs patients with blood collection every 6 mths in a 4-year follow-up, and evaluation of the immunological response to MCPyV capsid protein and oncoprotein antigens in each time-point. The project afterwards delved into the molecular characterization of MCC tissues, including investigations into MCPyV DNA and gene expression analyses.

Results

Sera (n=1900) sera from ADPs (n=650) were collected at different 6-mths time points, throughout a 4-year follow-up. ADPs, predominantly rheumatic disease- and Ankylosing spondylitis-affected patients, started a treatment with biologic drugs at baseline. Control sera (n=1089) were collected from healthy subjects (HS). Two immunoassays for detecting IgGs to MCPyV capsid proteins and oncoproteins were developed/validated on control sera from MCC patients (n=150) and HS. While the majority of ADP and HS sera were negative for anti-oncoproteins IgGs, lower rate of anti-capsid IgGs were determined in ADPs compared to HS. A consistent decline in the immune oncological response, i.e. IgGs against viral capsid antigens, was observed from early to late time points in biologics-treated ADPs, particularly among those ADPs receiving methotrexate and hydroxychloroquine. Next, MCC tissues (n=71) and 4 MCC cell lines (n=4) were molecularly analyzed. The majority of MCC were MCPyV-positive and expressed RASSF1/F5/F7, HLA-A, MICA/B, TAP1/2, LMP2/7 genes, together with miR-210-3p and miR-34a-5p microRNAs. No correlations were found between MCC patients clinicopathological information and MCPyV/gene expression data. We next

evaluated the methylome profile and retinoid gene signature in MCC cells. Hierarchical clustering and PCA analyses identified a strong separation of MCPyV-positive from MCPyV-negative MCC cells. SOX2, ISL1, and PAX6 were identified as MCPyV-associated upregulated hub genes.

Conclusions

Our data suggest that ADPs under immunosuppressive therapy might be prone to an impairment of the anti-MCPyV immune response, which might potentially lead, in turn, to an enhanced MCPyV replication. Our data may support the hypothesis that ADPs under immunosuppressive therapy may be predisposed to MCPyV infection, possibly as a consequence of the reduced ability to present MCPyV antigens thus favouring the MCPyV multiplication.

Francesca Sacco

Università degli Studi di Roma "Tor Vergata", Roma

Deciphering drug resistance mechanisms in FLT3-ITD positive acute myeloid leukemia

Background and hypothesis

The insertion site of the internal tandem duplications (ITDs) in the FLT3 gene affects the sensitivity to tyrosine kinase inhibitors (TKIs) therapy in acute myeloid leukemia (AML). Patients with the ITD in the tyrosine kinase domain lack effective therapeutic options. Here we tested the hypothesis that that the ITD insertion site causes a profound rearrangement of the cell signaling network, which in turn impacts the therapeutic potential of FLT3 inhibitors and chemotherapy to eradicate AML cells.

Aims

Here we aim at developing genotype-specific predictive signaling models and use these models to identify novel therapeutic strategies, based on the intentional rewiring of signaling pathways, to kill AML cells by combinatorial therapy.

Experimental design

State-of the art mass spectrometry-based (phospho)proteomics, interactomics and trascriptomics was integrated with network modelling techniques to derive genotype specific mathematical models. These models were used as a framework to simulate, in silico, which chemical perturbations increase AML sensitivity to apoptotic stimuli.

Results

The approach revealed a conserved role of the WEE1-CDK1 axis in TKIs resistance. Remarkably, pharmacological inhibition of the WEE1 kinase synergizes and strengthens the pro-apoptotic effect of TKIs therapy in cell lines and patient-derived primary blasts.

Conclusions

Finally, we propose a new molecular mechanism of TKIs resistance in AML and suggest the combination of WEE1 inhibitor and TKI as a therapeutic option to improve patients clinical outcome.

Valentina Serafin

Università degli Studi di Modena e Reggio Emilia, Modena

NFATc1 and NFATc2 control glucocorticoid resistance in pediatric T-cell acute lymphoblastic leukemia by regulating cholesterol biosynthesis and WNT/β-catenin pathway

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Background and hypothesis

Resistance to Glucocorticoids (GCs) is still a limitation in the treatment of pediatric T-cell Acute Lymphoblastic Leukemia (T-ALL) patients and a well-defined poor outcome predictor. Thus, the comprehension of GC resistance underlying mechanisms could improve T-ALL patients' overall survival. Interestingly, our research group has already unveiled the LCK kinase's pivotal role in T-ALL cells' GC resistance onset, although the downstream regulated biological processes remained to be elucidated. To this end, here we focused on the LCK downstream NFAT family transcription factors.

Aims

We aim to identify new GCs resistance mechanisms driven by LCK/NFAT pathway that can represent novel therapeutic targets paving the way to alternative therapeutic approaches to prevent or overcome GCs resistance and ameliorate the outcome of this subgroup of patients.

Experimental design

To identify the NFAT family members modulating GCs resistance we performed in vitro and in vivo proliferation assays in NFATs silenced or overexpressing T-ALL cells treated with GCs. Next, transcriptome analysis in NFATc1 or NFATc2 knock down cells allowed to infer the NFATc1 or NFATc2 driven biological processes responsible for GCs resistance. Moreover, by Nuclear Magnetic Resonance and Chromatin Immune Precipitation we characterized the lipidomic landscape in NFATc1 knock down cells and the NFATc1 or NFATc2 direct target genes.

Results

We demonstrated that exclusively NFATc1 or NFATc2 specific gene silencing restores GCs sensitivity in T-ALL GCs resistant cells, whereas their overexpression in GCs sensitive cells restores the resistance. Furthermore, we revealed that NFATc1 confer GCs resistance by directly regulating the transcription of cholesterol biosynthesis' genes. In agreement, exogenous cholesterol addition to NFATc1 knock down cells rebuild GCs resistance, on the contrary simvastatin sensibilizes T-ALL cells to GCs. Besides, we revealed that NFATc2 sustains GCs resistance by directly controlling the transcription of LRP6, a Wnt/β-catenin pathway player. Interestingly, the Wnt/β-catenin signaling activation restores GCs resistance in NFATc2 knock down cells, whereas its inhibition increases GCs sensitivity. Finally, we revealed that NFATc1 and NFATc2 promote GCs resistance by hindering the Glucocorticoid Receptor (GR) transcriptional activity. In agreement, diagnosed pediatric GCs resistant T-ALL patients display a high NFATc1-NFATc2 and a low GR transcriptional activity.

Conclusions

Overall, the identification of NFATc1 and NFATc2 as new regulator of GCs resistance through the modulation of cholesterol biosynthesis, Wnt/β-catenin signaling and GR transcriptional activity, will provide the rationale

for alternative therapeutic options to overcome T-ALL GCs resistance.

Tiziana Vaisitti

Università degli Studi di Torino, Torino

Unraveling pathogenic mechanisms in Richter's syndrome to open for translational opportunities

Background and hypothesis

Richter's syndrome (RS) is a rare condition arising from the transformation of chronic lymphocytic leukemia (CLL) into a more aggressive lymphoma with a rapidly fatal clinical outcome. The available therapeutic strategies are not satisfactory, resulting in low efficacy and high mortality, making RS a drug-demanding disease.

The driving hypothesis of the study is that by integrating different layers of exploration of the disease is possible to define a hierarchy of detrimental events and to identify novel targets for tailored therapeutic approaches.

Aims

The aim of this study is to shed light on some of the "dark sides" of the disease with two main goals. From the basic standpoint, the aim is to define the genetic, transcriptomic, and metabolic profile of RS cells to identify the molecular mechanisms involved in and driving RS pathogenesis. From the translational standpoint, the aim is to identify and pre-clinically validate potential therapeutic targets.

Experimental design

To address these questions, the study was divided in several complementary tasks, taking advantage of both primary RS samples and patient-derived xenograft (PDX) models. The planned tasks were intended to i) define the RS genetic fingerprint by DNA sequencing; ii) characterize the transcriptomic profile of RS cells with the identification of up- and down-regulated genes compared to CLL; iii) analyze the metabolic features of RS cells to understand the substrates dependencies and the main metabolic pathways these cells rely on; iv) provide a proof-of-concept of RS targeting by novel strategies.

Results

The genetic analysis of both primary RS and PDXs showed a high degree of heterogeneity among samples, with the identification of not previously reported genetic variants and confirmation of recurrent lesions. Moreover, several copy number variants were identified, affecting critical pathways such as DNA repair mechanisms, G2/M checkpoint, and the PI3K/AKT pathway. The transcriptomic profile of the same samples, and a comparison with the preceding disease phase, showed a sharp separation between RS and CLL, with up-regulation of genes involved in cell proliferation and metabolism.

In line with transcriptomic data, a functional analysis of the activity of several key enzymes belonging to different pathways highlighted that RS cells have a distinct metabolic profile compared to CLL cells, relying more on oxidative phosphorylation and amino acids metabolism, and less on fatty acid dismantling. In line with these results, glucose and glutamine uptake were increased in RS cells. Moreover, aggressive CLL samples showed a metabolic profile closer to RS samples, suggesting a progressive shift in metabolic features during transformation. A metabolomic analysis confirmed these characteristics highlighting the

activation of a more energetic pathway, as well as a shift towards the pentose phosphate pathway allowing for nucleotides production, critical to sustain RS proliferation.
Finally, based on genomic/transcriptomic/metabolic data, we explored novel targeting strategies based on both selective inhibitors, targeting the PI3K/NF-kB pathways, as well as on antibody drug conjugates capable of binding tumor associated antigens and delivering the payload selectively on RS cells.

Conclusions

These results show that matching data from different "omic" layers allows to identify RS pathogenic mechanisms and to design novel effective therapeutic strategies.

Claudio Vernieri

IFOM - Istituto Fondazione di Oncologia Molecolare, ETS, Milano

Exploiting inducible metabolic vulnerabilities to improve chemotherapy efficacy in triple negative breast cancer

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Background

Triple-negative breast cancer (TNBC) is the most aggressive and deadly subtype of breast cancer (BC). Dysregulation of glucose metabolism and growth factor-mediated signalling has been shown to sustain TNBC bioenergetics and anabolic functions. Consistently, combining cycles of fasting or fasting mimicking diets (FMDs) with cytotoxic chemotherapy produced synergistic anticancer effects in TNBC murine models. We recently showed that the FMD is well tolerated by cancer patients, and induces a significant reduction of blood glucose and growth factor concentration, combined with a previously undetected increase of plasma branched chain amino acids (BCAAs). Preliminary in vitro data indicate that modulating the ratio between extracellular BCAAs can be toxic to several tumor models including TNBC.

Hypothesis

We hypothesize that inhibiting metabolic pathways that are activated by TNBC cells in response to nutrient starvation/BCAA modulation can enhance the anticancer activity of starvation. Identifying those pathways will allow the design of synthetic lethal metabolic approaches to target TNBC metabolic heterogeneity.

Aims

To test our hypothesis, we will produce experimental growth media, collectively referred to as fasting-mimicking conditions (FMCs), which recapitulate FMD-induced metabolic changes, namely reduced glucose/growth factor and increased BCAA concentration. We aim to: 1) identify biological mechanisms responsible for the in vitro anti-TNBC activity of FMCs, alone or in combination with cytotoxic agents; 2) study FMC-induced modulation of crucial metabolic pathways, and the impact of downregulating these pathways on the anticancer effects of starvation; 3) unveil mechanisms through which an imbalanced concentration of extracellular BCAAs is toxic to TNBC cells.

Experimental design

Different human TNBC cell lines will be grown in standard media, and then switched to FMCs, alone or in combination with doxorubicin or cisplatin. We will study the impact of these treatments on cell survival, PI3K/AKT/mTORC1 axis activation and stress response pathways. To identify pathways of resistance to FMCs, we will perform global gene expression and proteomic analyses. Selected metabolic transporters/enzymes that are upregulated during FMCs will be downregulated in in vitro and in vivo (mouse xenografts) TNBC models, and cancer cell survival/tumor growth will be assessed. Crucial results of preclinical experiments will be validated in tumor specimens from TNBC patients undergoing the FMD, plus/minus metformin, in combination with standard chemotherapy in the context of a prospective clinical trial. Finally, we will characterize the molecular mechanisms mediating the cytotoxicity of BCAA imbalance in TNBC cells.

Expected results

Through a combination of population and time-lapse microscopy experiments, as well as of confirmatory in vivo experiments and analyses in tumor specimens from patients, we will identify new synthetic lethal anti-

TNBC metabolic interventions to enhance the anticancer activity of starvation. We will also elucidate the molecular mechanisms responsible for the cytotoxic effects of imbalanced extracellular BCAA concentration, and we will provide first time validation of this approach in animal experiments.

Impact on cancer

This project will elucidate metabolic pathways that TNBC cells activate to survive nutrient starvation, actually a highly promising, experimental anticancer approach. By identifying synthetic lethal metabolic interventions able to target intratumor and intertumor TNBC metabolic heterogeneity, this study will reveal new metabolic anticancer interventions to be tested in future clinical trials.

POSTER PRESENTATION

Vincenzo Amendola

Università degli Studi di Padova, Padova

Augmenting the therapeutic window of radiotherapy: biodegradable nanomedicine for imaging-guided RT enhancement

Background and hypothesis

Radiation therapy is used in about 50-70% of all cancer patients. Yet, in many circumstances, the efficacy is limited by the tolerance of normal tissues adjacent to the tumor, which affects cancer recurrence and patients' survival rate. However, the therapeutic index of radiotherapy can be increased by enhancement of radiosensitisation in tumor tissue and better confinement of the deposited dose to the tumor volume.

Aims

The project aims at realizing a new methodology to enhance efficacy and decrease toxicity of radiotherapy, hence, increasing the overall therapeutic window. The methodology relies on the integration of current radiotherapy and imaging protocols with a biodegradable gold-based theranostic nanomedicine. The theranostic nanomedicine will act as radiosensitizer and bimodal contrast agent for magnetic resonance imaging (MRI) and computed x-ray tomography (CT), and will be biocompatible, easily producible, capable of passive or active tumor targeting and, finally, biodegradable.

Experimental design

The research plan consists of a multidisciplinary and circular workflow going from the theoretical optimization of the theranostic nanomedicine to its synthesis and characterization, to in vitro experiments of the theranostic efficacy, to the in vivo verification of effects on tumor biology and preclinical assessment of the extension of the therapeutic window. Prostate cancer is used as a representative model of tumors treated with radiotherapy.

The theranostic agent is developed starting from an already published and patented 4-D transformable Au-Fe alloy nanomedicine. Modelling and experimental investigations will guide to the maximization of the nanomedicine performances, exploiting the flexibility, low-cost, rapidity and scalability of our laser-assisted synthesis protocol.

The nanomedicine is first tested and optimized in vitro for biocompatibility, biodegradability, stability, passive or active targeting, imaging and radiosensitization efficacy. The optimized nanomedicine will subsequently be tested in vivo by intravenous administration or local delivery through injectable gels, to assess tumor targeting ability, imaging-guided radiosensitization and clearance after use.

Results

So far, a set of nanomedicines acting as radiosensitizers have been produced, tested and refined. The compositional and structural features of the nanomedicines have been investigated and understood as a function of the type and ratio of elements, which include gold, iron, silver and boron. The in vitro tests of the nanomedicines indicated that several formulations are theranostic nanosystems biodegradable, biocompatible, x-ray radiosensitizers, and bimodal contrast agents for MRI and CT. Some formulations retained the theranostic functions but are not biodegradable. For the formulation based on gold and iron, we have shown that tumor cells (PC3) and highly proliferating cells (HEK) are more sensitive than fibroblasts

to the nanoparticles also in the absence of X-rays. The formulations containing boron showed perspectives for the use of bot X-rays and neutron capture radiotherapy approaches (XRT and BNCT), towards the more effective treatment of normal and hypoxic tumor regions.
Finally, a protocol for the verification of nanoparticles uptake by tumor cells, and its relation to the radiosensitization effects, has been developed, using pure gold nanoparticles as benchmark and wisely applying batch (ICP-MS) and single cell (electron microscopy) analysis. This information resulted crucial for designing the in vitro experiments and properly assessing the effectiveness of the nanomedicine as radiosensitizers.

Conclusions

A set of biodegradable nanomedicines has been developed and studied according to the project workplan, towards the assessment of the ability to expand the therapeutic window of XRT. Model systems have been also developed and studied to improve the understanding of the nanomedicine features and improve the engineering capabilities, as well as to identify reliable experimental procedures for the assessment of their functions. The nanomedicines do increase the radiation effects in vitro and have selective citotoxic effects on tumor cells and rapidly proliferating cells compared to normal cells. Besides, the nanomedicines developed so far have opened additional perspectives in the synergistic use of radiotherapy techniques which act on bot normal and hypoxic tissues.
The project is prosecuting with the identification of a protocol based on the administration (intravenous or by local gel injection) of the theranostic nanomedicine for imaging-guided radiation planning, which can expand the current therapeutic window of radiotherapy.
From a future perspective, the results will impact also on therapeutic and diagnostic potentialities of emerging technologies such as MRI-linac, proton radiotherapy or x-ray fluorescence CT.

Valentina Audrito

Università degli Studi del Piemonte Orientale "Amedeo Avogadro", Alessandria

The impact of NAMPT/NAD axis in metabolic/translational reprogramming and tumor-host crosstalk in metastatic melanoma.

Background and hypothesis

The frequent emergence of drug resistance in BRAF-mutated metastatic melanoma (MM) patients remains a challenge. Resistance is coupled by a set of rewiring processes in both tumor and immune cells. MM cells showed increased levels of the redox cofactor NAD, essential to support their metabolic adaptation underlying the acquisition of drug resistance. This was obtained selectively overexpressing the rate-limiting NAD-biosynthetic enzyme nicotinamide phosphoribosyltransferase (NAMPT). NAMPT-NAD axis becomes a driver of melanoma progression and resistance to targeted-therapy.

The hypothesis behind this part of MFAG project is that NAMPT/NAD axis in addition to promote metabolic rewiring in resistant melanomas, may impact also on translational reprogramming and immune regulation, two other processes involved in the mechanisms of acquired resistance to targeted-therapy and immune checkpoint inhibitors (ICIs).

Aims

This part of MFAG proposal aims at dissecting i) the interplay between NAD/NAMPT and mammalian target of rapamycin (mTOR) signaling axes in regulating translation in sensitive and resistant melanoma cells and ii) a novel reciprocal regulation between NAMPT and interferon-gamma (IFN-γ)-mediated signaling activation involving PD-L1, linking NAMPT-dependent metabolic reprogramming and immune regulation.

Experimental design

The study was organized in two experimental lines addressing the two aims described. We used multiple and complemental experimental strategies and methods, as described in the results, exploiting different BRAF-mutated human melanoma cell lines sensitive (S) and resistant (BiR) to BRAF (i)nhibitors (BRAFi), previously generated.

Results

Regarding Aim 1 we showed that NAMPTi (FK866, OT-82) induce a translational arrest in melanoma cells S and BiR. This was paralleled by the activation of the energy sensor 5' AMP-activated protein kinase (AMPK) and the inhibition mTOR/4EPB1 pathway impacting on the formation of eIF4F and eIF2 complexes, key initiation factors in the cap-dependent mRNA translation. All these events lead to protein synthesis arrest, that we measured directly using Click-it chemistry based on the incorporation of an aminoacid analog (AHA). Polysome fractionation highlighted changes in polysome profiles when cells were treated with OT-82. Lastly, on 80s and pooled heavy and light polysome fractions we were able to detect NAMPT protein by western blot, suggesting the potential association of this enzyme to ribosomal proteins or initiation factors, as also emerged by the cellular NAMPT interactome obtained by NAMPT immunoprecipitation (IP) following mass-spectrometry.

Regarding Aim 2 analyzing the TCGA melanoma cohort and cell lines database we found a positive and

significant correlation between NAMPT expression and IFN-γ signaling. Focusing on PD-L1, a target of IFN-γ, we revealed a direct correlation with NAMPT at protein level, as demonstrated by analyzing a melanoma tissue microarray. Melanoma cells treated with IFN-γ upregulate NAMPT, and vice versa, NAMPTi markedly reduce the activation of the IFN-γ signaling and PD-L1 expression. Preliminary data revealed the downregulation of NAMPT expression in the presence of JQ1, a Bromodomain and Extra-Terminal motif (BET) protein inhibitor, suggesting an epigenetic regulation of NAMPT, like for PD-L1, that will be further investigated.

Conclusions

Overall, these data highlighted i) a potential direct impact of NAD/NAMPT axis in translational reprogramming, and ii) a reciprocal regulation between NAMPT and IFN-γ/PD-L1 signaling activation potentially impacting on response to ICIs.

Isaia Barbieri

Università degli Studi di Torino, Torino

The RNA 5' cap methyltransferase TGS1 regulates redox metabolism in Acute Myeloid Leukaemia.

Background and hypothesis

RNA modifications represent a new epigenetic mechanism controlling gene expression and are involved in the pathogenesis of several diseases, including cancer. RNA modifications are specifically required for the growth of cancer cells and pharmacological inhibition of RNA modifying enzymes emerged as a promising approach for cancer therapy.

Aims

Our main goal is to investigate the roles of RNA modification enzymes in cancer and to characterize them as potential therapeutic target.

Experimental design

We previously identified Trimethylguanosine synthase (TGS1) as one the top target required for AML cell proliferation through a CRISPR dropout approach. TGS1 is responsible for the conversion of the m7G-cap to 2,2,7 trimethylguanosine (m2,2,7G) on specific RNAs. Firstly, we validated our in several human AML cell lines by shRNA interference. Our cellular models will be used to identify the biological functions and molecular mechanisms mediated by TGS1 activity and m2,2,7G hypermethylation in AML.

Results

By developing and performing m2,2,7G RNA-IP-seq, we were able to identify TGS1 direct targets. Our RNA-IP analysis identified more than 500 modified mRNAs which are highly enriched for nuclear genes encoding for mitochondrial proteins, especially members of the oxidative phosphorylation pathway. While mRNA levels of these targets are not affected by TGS1-KD, protein levels are significantly downregulated. Molecularly m2,2,7G promotes the translation of TGS1 target genes through its interaction with the non-canonical translation initiation factor EIF3b

Effects of TGS1-KD on AML cells metabolism were characterized by metabolites Mass Spectrometry (MS) analysis and by profiling the energetic and redox state of targeted cells. We observed an increased oxidative stress in TGS1-depleted AML cells, characterized by high level of cellular Reactive Oxygen Species (ROS) and by the upregulation of ATF4 protein level. Despite TGS1-KD impairs the expression of the antioxidant enzyme GPX4, we couldn't observe any consistent lipid oxidation upon TGS1-silencing, which could potentially trigger ferroptosis of targeted cells. Nevertheless, TGS1-targeted cells are sensitised to sub lethal doses of a variety of metabolic drugs, including ferroptosis-inducing agents.

Conclusions

We identified TGS1 as master regulator of cellular oxygen metabolism in AML cells and demonstrated that its targeting represents a potential new strategy to treat leukaemia patients.

Eduardo Bonavita

Humanitas Mirasole S.p.A., Rozzano

Analysis of mechanisms of immune evasion in liver cancer

Background and hypothesis

The immune milieu of the liver maintains a delicate balance between activation against pathogens and tolerance toward microbiota or food-derived antigens. However, this tolerogenic nature hinders the development of an effective anti-tumor immune response. Hepatocellular carcinoma (HCC) stands as a leading cause of cancer-related deaths globally. Surgical intervention is often unfeasible for most patients, and resistance to immunotherapy is frequently observed.

Aims

Our objective is to elucidate the contribution of various immune populations in shaping the anti-tumor response in different liver cancer contexts. Given their pivotal role in initiating immune responses and promoting immune tolerance, our focus will be on conventional dendritic cells (cDC), specifically activated/mature cDC (mDC).

Experimental design

mDCs possess the ability to migrate to lymph nodes, where they cross-present tumor antigens and secrete IL-12, thereby orchestrating cytotoxic anti-tumor responses. We aim to leverage two recently developed gene-targeted mouse models, enabling the tracking and inducible depletion of mDCs to investigate their role in HCC. For this purpose, we have established autochthonous HCC models in which tumors can be induced either genetically or through treatment with carcinogens.

Results

Importantly, we are exploring how diverse genetic makeups of HCC influence the tumor immune infiltrate during progression. Ultimately, by dissecting the intricate interplay of immune populations and employing cutting-edge mouse models, in vitro platforms, and advanced cellular and molecular techniques, this research aims to deepen our understanding of HCC immunopathology.

Conclusions

The insights gained have the potential to inform innovative strategies for overcoming resistance to current therapeutic interventions, ultimately enhancing the effectiveness of anti-tumor immunity in liver cancer.

Federica Cossu

Consiglio Nazionale delle Ricerche, Milano

Targeting BIR-mediated onco-PPIs: rational design of NF-κB modulators

Background and hypothesis

The over-expression of Inhibitors of apoptosis proteins (IAPs) family enhances cell survival and resistance to chemotherapics. IAPs-mediated complexes ubiquitylate substrates thus regulating NF-κB pathway. Type I BIR (Baculovirus IAP repeat) domains of IAPs are pivotal for the assembly of such complexes. IAPs' Type-II BIRs directly interact with caspases to inhibit cell-death, or with SmacDIABLO for apoptosis restoration. Type II BIRs-directed therapies, Smac-mimetics (SMs), relieve caspases from inhibition by X-linked IAP and induce cIAPs (cellular IAP1 and 2) auto-ubiquitination and degradation. BIR-mediated Protein-Protein Interactions (PPIs) are validated onco-targets.

IAPs-directed therapies are designed targeting pockets or hotspots on isolated, globularly structured BIR domains. However, BIRs relative positioning within the entire IAP molecule is the key for various pro-survival roles. Besides our, few groups managed to isolate full-length IAPs obtaining the yields required for structural studies. SMs, now in advanced phases of clinical trials, produce divergent effects, as observed in SMs-resistant cancer cell lines, where cIAP2 is upregulated promoting cell survival. Information about the structural details of full length IAPs is necessary. In fact, SMs were hypothesized to induce huge IAPs re-arrangements, but it has not been fully demonstrated yet. Furthermore, we identified compounds binding BIR1-mediated PPI surfaces, showing disruption of IAPs-containing complexes and IAPs-dependent cell-toxicity.

Aims

The project targets BIR-mediated onco PPIs to (i) develop/improve IAPs-targeting therapies and (ii) unravel the molecular determinants of the action of IAPs or IAPs-inhibitors. To this purpose, we aim at identifying FL-IAPs constructs suitable for structural analysis, also in the presence of partners and ligands. Furthermore, structure-driven and bio-chemical/-physical approaches will allow the selection of modular IAPs-selective molecules, ultimately tuning NF-κB.

Experimental design

We propose to expand the chemical space around the lead Cmp2, generating a new class of potential cancer therapics. Furthermore, we will consider the synthesis of hybrid compounds, coniugating anti-BIR1 compounds with SMs. We plan to investigate the triggered cellular pathways on a large panel of tumoral cell lines and characterize the biophysical properties underlying their action. Particular attention will be paid to cells described as resistant to IAPs-antagonists or other chemotherapeutic agents after IAPs activation. The structure-based approach will reveal details on protein-ligands interactions and consequent conformational changes of IAPs, providing the rationale for drug lead optimization. Furthermore, the virtual screening of compounds libraries against the new IAP structural hotspots will expand the number of chemicals to be included in the development of new anti-cancer candidates.

Results

We characterized a library of more than 50 novel putative anti-cancer molecules in vitro, analysing the ability

to bind target proteins and to induce cell death in a panel of four tumor cell lines (prostate cancer, triple negative adenocarcinoma, non-small cell lung cancer). We selected 2-3 candidates with best in vitro profiles for further experiments, to elucidate pro-death mechanisms. We are producing the recombinant form of different FL-IAP homologue and protein partners as TRAF2 and TAB1. A deeper understanding of the action of IAPs in the presence/absence of selected drug candidates at the molecular level and the use of high-resolution techniques represent steps forward the development of optimised treatments.

Conclusions

The modulation of pro-survival complexes regulating the NF-κB pathway, as the ones mediated by IAPs, can be the strategy to overcome cases of resistance to current IAPs-targeting chemotherapics, to better define IAPs roles/functioning and find accurate/selective therapies.

Antonio De Leo

Alma Mater Studiorum Università di Bologna, Bologna

Tumor microenvironment in endometrial carcinomas: the emerging role of spatial cancer-immune phenotypes and HLA class I expression

Background and hypothesis

Compartmentation of the immune response into three main Spatial Cancer-Immune phenotypes (SCIs) - inflamed, excluded, and desert - has been proposed as prognostic parameter and as potential predictor of response to immunotherapy.

Aims

The aim of the study is to characterize the SCI and HLA-I expression in endometrial carcinomas (EC) by correlating them with molecular subtypes, immune gene expression profiles, and prognosis.

Experimental design

Immunohistochemistry (IHC) and Next-Generation Sequencing (NGS) are used to assign TCGA molecular EC subgroups: POLE mutant (POLE), mismatch repair deficient (MMRd), p53 mutant (p53abn), and no specific molecular profile (NSMP). IHC expression of HLA-I, CD20, CD3, CD8, PD-1, PD-L1, CD68 has been assessed and quantified by digital image analysis on whole tumor tissue sections.

Results

A total of 213 ECs were stratified into four molecular subtypes: 17 (8.0%) POLE, 68 (31.9%) MMRd, 42 (19.7%) p53abn, and 86 (40.4%) NSMP. SCI determination showed 105 (49.3%) inflamed, 62 (29.1%) desert, and 46 (25.6%) excluded tumors. The inflamed phenotype was more prevalent in MMRd (64.7%) and POLE (76.5%) subtypes compared to NSMP (45.3%) and p53abn (21.4%) (P-value<0.001). The inflamed phenotype was more prevalent in MMRd (64.7%) and POLE (76.5%) subtypes compared to NSMP (45.3%) and p53abn (21.4%) (P-value<0.001). The prognostic effect of SCIs was dependent on the context of the molecular subtype. While the excluded SCI was significantly associated with shorter disease-free survival in the entire cohort (log-rank P =0.011), in a stratified analysis by molecular subtype was only strongly prognostic within the NSMP subtype (log rank P <0.001) and remained significant in early stage NSMP tumors. We also assessed the IHC expression of human leukocyte antigen class I (HLA-I), which plays an important role in the regulation of the immune response. In the entire cohort, HLA-I loss was statistically associated with adverse prognostic parameters, with excluded phenotype, and disease recurrence. In NSMP tumors, that represent the most heterogenous EC subgroup, HLA-I loss was independently associated with reduced disease-free survival. Gene expression analysis confirms different immune signatures based on the immune features identified (SCIs and HLA-I).

Conclusions

The results highlight that the different patterns of immune response and HLA-I expression may represent new prognostic parameters in the NSMP subtype. The integration of these features could be a promising opportunity to improve the prognostic risk stratification of patients and may guide the therapeutic approach, particularly in the NSMP subtype.

Carminia Maria Della Corte

Università degli Studi della Campania Luigi Vanvitelli, Napoli

DNA-PK inhibition sustains anti-tumour innate immune response in SCLC

Background and hypothesis

Small cell lung cancer (SCLC) is challenging to manage because of its high aggressiveness. The combination of DNA damaging therapies (chemotherapy, radiotherapy, or DNA damage repair inhibitors, DDRi) with immunotherapy (IO) enhances the anti-tumor immune response via activation of the Stimulator of Interferon Genes (STING). However, only a small subset of SCLC, defined as "inflamed," responds to immunotherapy, and novel strategies to improve immune responsiveness are needed.

Aims

We hypothesized that DDRi may induce simultaneous activation of multiple innate immune pathways (STING and MAVS), thereby sensitizing otherwise immunoresistant SCLC.

Experimental design

We used three different models of SCLC, specifically SCLC cell lines, SCLC patient PBMCs (pre- and post-chemoimmunotherapy, CIT), and 2D/3D co-cultures of SCLC cells and SCLC patient PBMC subsets, to assess the effect of the DNA-PK inhibitor, a novel DDRi, on the secretion of inflammatory chemokines, immune-mediated cytotoxicity, and metabolism.

Results

The expression of STING and mitochondrial AntiViral Signalling protein (MAVS) was significantly upregulated in DNA-PKi-treated SCLC cells and patient-derived PBMCs. A novel mechanism of STING mitochondrial recruitment and its physical interaction with MAVS were discovered in DNA-PKi-treated immune cells. DNA-PKi affected the metabolism of immune cells, polarization of PBMC-derived monocytes towards an anti-tumor M1 phenotype, and improved in vitro NK cell-mediated cytotoxicity. Finally, 3D co-culture models showed increased lymphocyte infiltration of tumor spheroids after DNA-PKi treatment, with consequent tumor structure disruption.

Conclusions

We uncovered a novel STING-MAVS interplay as a mediator of the anti-tumor immune response induced by DNA-PKi, thus proposing DNA-PK inhibitors as a novel therapeutic strategy to improve the IO response in SCLC.

Giuseppe Ercolano

Università degli Studi di Napoli "Federico II", Napoli

Deciphering the Role of Innate Lymphoid Cells (ILCs) in Prostate Cancer patients.

Background and hypothesis

Prostate cancer (PCa) is one of the most common cancer in males worldwide with a high mortality rate. Different immune cell populations are associated with PCa progression, therapy resistance and establishment of a pro-tumoral microenvironment. Innate lymphoid cells (ILCs) represent a recently identified family of innate immune cells considered as the innate counterpart of T lymphocyte subpopulations. In particular, type-2 ILCs (ILC2s) are defined as a primarily pro-tumorigenic subset through the production of type 2 cytokines (IL-5, IL-13). Nevertheless, nothing is known about the contribution of ILCs in PCa development and progression.

Aims

In this study we characterized the role of ILCs in terms of frequency and function in PCa in the Peripheral Blood Mononuclear Cells (PBMCs) of PCa patients.

Experimental design

PBMCs were isolated by Lymphoprep. PCa patients (n= 43) were classified in low-grade and high-grade (LG and HG respectively) according to the Gleason score and ILCs frequency was evaluated by flow cytometry and compared to healthy donors (HDs) (n= 21). The levels of ILC2-activating cytokines have been analyzed by multiplex assay in the serum of HDs and PCa patients (n=8 and n=24 respectively). For evaluating the crosstalk between ILC2s and cancer cells, PC3 human prostate cancer cells were used. Finally, by exploiting bioinformatical tools, we evaluated the impact of ILC2 in PCa patients survival as well as the contribution of IL-33 and IL-18 in sustaining the progression of PCa.

Results

We found that the frequency of ILC subsets was dysregulated in PCa patients. In particular, ILC2s were increased in both LG and HG PCa patients. The highest frequency of ILC2 was correlated with the Prostate-Specific Antigen (PSA) levels in PCa patients and affected patients' survival. Moreover, the frequency of ILC2s in PCa patients was correlated to higher levels of ILC2-activating cytokines revealing that both IL-33 and IL-18 might play a potential role in PCa progression.

Conclusions

Our results suggest that both ILC2s and their activating cytokines (IL-33 and IL-18) could represent a novel therapeutic target for the treatment of PCa.

Giorgia Foggetti

Università Vita-Salute San Raffaele, Milano

Unveiling Novel Therapeutic Targets in Lung Adenocarcinoma

Background and hypothesis

Targeted therapies dramatically improved the survival and life quality of patients with lung adenocarcinoma. However, although the overall efficacy of targeted therapies is high, so is the variability in the depth and duration of drug response, which ultimately leads to the onset of resistance. A big challenge in the field is to investigate specific features of tumor subsets that are less sensitive to standard therapy to define personalized approaches.

Aims

By leveraging our institutional translational program, we aim at generating a cohort of unique preclinical models of lung cancer that mimic the heterogeneity of responses to targeted therapy (i.e., heterogeneity of response to osimertinib in EGFR mutant lung adenocarcinoma). Specifically, we will define subsets of models that display a reduced response or are intrinsically resistant to therapy and investigate the molecular mechanisms that are associated with a non-sensitive phenotype. Importantly, we will correlate our findings with in vivo data and combine them to clinical data to identify potential therapeutic targets. Finally, we will test the efficacy of personalized strategies in our settings extending these experiments to drug-resistant models that we developed in the Lab to evaluate whether a specific drug combination may apply to additional lung cancer genotypes and phenotypes.

Experimental design

We aimed to establish patient-derived models including organoids, xenografts, and 2D cell lines from human specimens collected from patients with EGFR mutant lung cancer. As regards 3D model generation, we evaluated organoid formation and growth as well as histological features, propagate organoids up to three weeks of culture, and further expanded organoids upon passaging and dissociation. We profiled newly generated patient-derived models and evaluated sensitivity to standard therapy to stratify these in sensitive (S), less sensitive (LS), and intrinsically resistant (IR) tumors by performing viability assays. Ongoing efforts are devoted to establishing 2D cell lines and xenografts from the same tissue samples that were collected at the time of surgery.

Results

At this time, we enrolled 13 patients in ad hoc protocol to investigate EGFR mutant lung cancer, GINGER, with a ~70% of tissue availability to generate patient-derived models. Upon tumor dissociation, individual cells proliferated and formed spherical organoid morphology along with increasing metabolic activity that was maintained during the time of culture. Notably, patient-derived models recapitulated lung adenocarcinoma histological features matching the tissue of origin and expressing lung adenocarcinoma markers. Importantly, targeted therapy-naïve models displayed heterogeneity of drug responses thus reflecting clinical outcomes observed in patients and highlighting the relevance of our studies to identify subsets of tumors that might benefit from additional interventions.

Conclusions

In summary, over the past months we laid the foundations for our institutional translational program, focusing on the generation of patient-derived models of EGFR mutant tumors through the GINGER study with the potential and feasibility to extending our expertise to tumors with other oncogene addictions beyond EGFR. Future experiments with these models will provide insights into the first step for a structured precision–medicine program for patients with lung cancer to increase the translatability of research findings into the clinic, supporting faster progress toward long-lasting outcomes for patients with lung cancer.

Maria Rosaria Galdiero

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Theranostic roles for neutrophils and neutrophil extracellular traps in melanoma patients under checkpoint inhibitors

Background and hypothesis

Melanoma displays a rising incidence, and the mortality associated with metastatic form remains high. Monoclonal antibodies that block programmed death (PD-1) and PD Ligand 1 (PD-L1) network have revolutionized the history of metastatic disease. PD-L1 is expressed on several immune cells and can be also expressed on human neutrophils (PMNs). The role of peripheral blood PMNs as predictive biomarkers in anti-PD-1 therapy of melanoma is largely unknown.

Aims

In the first part of the project, we aimed at determine activation status and PD-L1 expression on human PMNs as possible biomarkers in stage IV melanoma patients (MPs). In the second part, we aimed at in vitro investigating the role of PMNs and their related mediators in human melanoma.

Experimental design

We prospectively recruited 65 patients with stage IV melanoma candidates for PD-1 inhibitors (nivolumab) at the Istituto Nazionale Tumori—IRCCS—Fondazione "G. Pascale" of Naples, Italy. Peripheral blood samples were collected and freshly processed at baseline and every 12 weeks. Blood samples of 42 healthy donors, sex and age-matched, were collected at the University of Naples Federico II, Naples, Italy. We prospectively investigated the frequency of peripheral blood PMNs by means of flow cytometry analysis. Serum samples were used to measure concentrations of a panel of cytokines and chemokines involved in activation and polarization of PMNs by ELISA.

Highly purified human PMNs from healthy donors (>99% purity) were stimulated in vitro with conditioned media (CM) derived from the melanoma cell lines SKMEL28 and A375 (melanoma CM), and primary melanocytes as controls. PMN biological properties (chemotaxis, survival, activation, cell tracking, morphology and NET release) were evaluated.

Results

We found that PMNs from MPs displayed an activated phenotype and increased PD-L1 levels compared to healthy controls (HCs). Patients with lower PD-L1+ PMN frequencies displayed better progression-free survival (PFS) and overall survival (OS) compared to patients with high PD-L1+ PMN frequencies. Multivariate analysis showed that PD-L1+ PMNs predicted patient outcome in BRAF wild type MP subgroup but not in BRAF mutated MPs.

We found that the A375 cell line produced soluble factors that promoted PMN chemotaxis, survival, activation and modification of morphological changes and kinetic properties. Furthermore, in both melanoma cell lines CM induced chemotaxis, activation and release of neutrophil extracellular traps (NETs) from PMNs. In contrast, the primary melanocyte CM did not modify the biological behavior of PMNs. The transcriptional profile of highly purified human PMNs stimulated in vitro with melanoma CM is going to be

evaluated by NGS and analyzed using adapted software. In addition, serum levels of myeloperoxidase, matrix metalloprotease-9, CXCL8/IL-8, granulocyte and monocyte colony-stimulating factor and NETs were significantly increased in patients with advanced melanoma compared to HCs.

Conclusions

PD-L1+ PMN frequency emerges as a novel biomarker in stage IV BRAF wild type MPs undergoing anti-PD-1 immunotherapy. Patients with metastatic melanoma display increased circulating levels of neutrophil-related mediators and NETs. In addition, melanoma cell lines produce soluble factors able to "educate" PMNs toward an activated functional state. Our findings add a piece in the puzzle of the multiple roles of neutrophils and their mediators in melanoma patients undergoing immunotherapy. Further investigations are currently ongoing to better understand the role of "tumor-educated neutrophils" in modifying melanoma cell behavior.

Paola Infante

Università degli Studi di Roma "La Sapienza", Roma

The cytoskeleton regulator inverted formin INF2 regulates the SHH pathway and medulloblastoma tumorigenesis

Background and hypothesis

Medulloblastoma (MB) is the most common malignant pediatric brain tumor. The high heterogeneity of MB makes extremely difficult determining a successful therapy. Among MB's molecular subgroups, Sonic Hedgehog (SHH) is the most abundant and it is characterized by alterations of key components of the SHH development pathway. However, the molecular mechanisms driving SHH-MB still require to further be unveiled to design more effective therapies. Recently, defects in cytoskeleton remodeling are emerging as an important hallmark of cancer. Here, we identified INF2, a formin involved in the regulation of actin and microtubule cytoskeletal dynamics, as a putative negative regulator of SHH signaling and SHH-dependent MB growth. We believe that the regulation of the SHH pathway mediated by INF2 represents a novel and relevant aspect for MB biology, which could illuminate on the role of cytoskeleton in SHH-driven tumorigenesis.

Aims

In this field of study, our main goals are: i) deciphering the role of INF2 in the regulation of SHH signaling; ii) characterizing the mechanisms by which INF2 controls SHH signaling; iii) studying the biological role of INF2 in SHH-dependent cell growth and SHH-MB tumorigenesis.

Experimental design

Luciferase functional assays, western-blot and RT-qPCR have been performed to analyze the effect of INF2 on GLI1 (the final effector of SHH signaling) transcriptional activity and expression. Proliferation assays and Brillouin microscopy have been carried out on primary murine SHH-MB cells to test the effect of INF2 modulation on SHH-MB growth and stiffness.

Results

We demonstrated that INF2 is a negative regulator of SHH signaling, with an opposite role previously described for mDia formin. The overexpression of INF2 counteracts the positive effects of mDia on GLI1 transcriptional activity and expression. Moreover, INF2 is highly expressed in late stages of murine cerebellum development when SHH signaling is switched off, showing an opposite trend to mDia. Accordingly, the INF2 genetic silencing increases the expression of GLI1 and the proliferation of granule neuronal progenitors (GNPs), the cells of origin of MB. Interestingly, INF2 protein levels were strongly reduced in SHH-MB samples, contrarily to mDia. Notably, the overexpression of INF2 in SHH-MB primary cells from Math1-Cre/Ptc1fl/fl mice significantly inhibits the cell proliferation as consequence of the reduction of GLI1 expression levels and increases the stiffness of primary SHH-MB cells, thus suggesting that INF2 could affect tumor cell motility and invasiveness.

Conclusions

Overall, our findings unveil INF2 as new player of the SHH pathway paving the way to study cytoskeletal

remodeling proteins as a novel area of investigation in SHH-MB for the design of innovative therapeutic interventions.

Donato Inverso

Università Vita-Salute San Raffaele, Milano

Spatially-resolved phosho-proteomics of NASH progression

Background and hypothesis

Non-alcoholic steatohepatitis (NASH) is part of a progressive metabolic dysfunction affecting a quarter of the global population, with projections indicating it as a primary precursor to hepatocellular carcinoma (HCC) development. The transition from initial fatty liver disease to HCC involves complex interplays and the gradual disruption of liver parenchyma organization. Recent insights into the "angiocrine" function of liver endothelial cells (L-EC) highlight their crucial role in determining the spatial arrangement of liver microarchitecture. However, the precise characterization of the L-EC contribution during NASH progression remains largely undetermined and faces limitations due to inherent challenges in correlating morphological information with multiomics data.

Aims

This project has three major focuses: to define a multiomic platform, providing functional and spatially resolved analyses of the hepatic lobule zonation during NASH and HCC progression; to provide a phosphorylation signature of NASH-to-HCC transition and of HCC heterogeneity, to identify signaling pathways that cannot be studied by conventional sequencing approaches; to dissect molecular mechanisms downstream to anti-VEGFRs therapy and their relative impact on immune-checkpoint inhibitors.

Experimental design

We utilized a spatial FACS-sorting strategy in conjunction with transcriptomics and quantitative phosphoproteomics. This approach provided the first draft of the anatomical organization of protein signalling within the liver vasculature. Notably, we also identified tyrosine phosphorylation, the major target of antiangiogenic therapy, as one of the most spatially regulated signalling events. The same approach was then applied to mice fed with choline-deficient high fat diet or western diet to recapitulate the entire spectrum of pathological changes observed in human patients, from simple steatosis to HCC.

Results

Our findings indicate that the widespread disturbance of endothelial zonation is an early event during NASH induction. Noticeably, Wnt9b, whose expression gradient defines the zonation signature of the adjacent hepatocytes, undergoes a complete loss of its normal zonation pattern. Consistently, NASH progression resulted in the disappearance of conventional patterns associated with zonation markers like glutamine synthetase in both murine models and human patients. Finally, we found a global reduction of the vascular tyrosine kinase activity including VEGFR, whose inhibition represents a first-line therapy for the unresectable HCC.

Conclusions

Our results align with recent clinical data demonstrating that combining anti-VEGF and anti-PD1 therapies, as opposed to anti-PD1 therapy alone, extends the survival of patients with advanced HCC. Our work emphasizes the idea that the loss of spatial organization of protein phosphorylation could be a pivotal

pathological event during the transition from NASH to HCC.

Sara Lovisa

Università Humanitas, Pieve Emanuele

Epithelial-to-Mesenchymal Transition at the crossroads of inflammation and cancer initiation

Background and hypothesis

Epithelial-to-Mesenchymal Transition (EMT), an embryonic program by which epithelial cells lose their identity and acquire mesenchymal features, is known to be relaunched in pathological conditions such as fibrosis and cancer. EMT has been traditionally associated with the generation of fibrosis-associated fibroblasts and with the promotion of metastasis in tumor cells, however in this past decade there has been a novel understanding of the functional role of EMT during pathogenic conditions. Specifically, EMT has been identified as an injury-induced response of damaged epithelial cells which impairs their regenerative potential and orchestrates the inflammatory and fibrogenic tissue response. Although the possible involvement of EMT in the initial steps of tumorigenesis has been suggested, whether EMT in functionally involved in the initiation of cancer arising from chronic inflammatory and fibrotic diseases is currently unknown. We have therefore hypothesized that EMT could participate in the early phases of inflammation-induced tumorigenesis by priming epithelial cells to develop pre-cancerous modifications.

Aims

Using the mammalian intestine as a model, we seek to unveil the functional role of EMT in tumor initiation by pursuing the following two specific aims. Aim 1: investigate the impact of EMT activation during acute injury and chronic inflammation. Aim 2: determine the role of EMT in inflammation-induced cancer emergence.

Experimental design

Novel mouse models that enables the genetic manipulation of EMT in the intestinal epithelial cells (IECs) through deleting the mesenchymal driving transcription factors Twist and Snail (Twist IEC-cKO and Snail IEC-cKO) have been generated to carry out this project. These mice also contain the CAG-LSL-tdTomato allele to lineage trace and fate map EMT cells. WT and cKO mice were challenged with acute and chronic IEC injury models by Dextran Sodium Sulfate (DSS) treatment for the purpose of Aim 1, and with the inflammation-induced tumorigenesis model by AOM/DSS protocol to induce colitis-associated colon cancer (CAC) for the purpose of Aim 2.

Results

Obtained results indicate that EMT-cKO mice display an accelerated recovery from acute intestinal injury as demonstrated by reduced body weight loss and ameliorated clinical signs of colitis. This phenotype was also paralleled in the chronic setting which led to the development of fibrosis. A gender-dependent difference was noted, with the male mice displaying a more pronounced phenotype. Time course analysis of EMT activation revealed partial EMT activation at the early phases of epithelial injury followed by the appearance of full EMT, both regressing during the recovery phase. When subjected to the well-established inflammation-induced tumorigenesis model, EMT genetic deletion impacted on tumor multiplicity and

tumor volume.

Conclusions

The collected observations clearly indicated EMT as an epithelial program functionally involved in the response to acute injury as well as impacting on fibrosis development and neoplastic transformation of the colonic mucosa. These results contribute to understanding the role of injury-induced epithelial plasticity in promoting long-term tissue rearrangements, i.e. fibrosis, and malignant transformation.

Nicola Manfrini

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Defining the intracellular networks involved in FAM46C oncosuppressor complex functionality

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Background and hypothesis

FAM46C has been recently identified by our group and others as a strong tumour suppressor in multiple myeloma (MM).

Our working hypothesis is that FAM46C is part of a high molecular weight protein complex that, by localizing at the Endoplasmic Reticulum (ER) thanks to interaction with ER-bound protein FNDC3A, works at the crossroads between protein stability, intracellular trafficking and secretion. Specifically, by altering vesicle dynamics, FAM46C affects protein secretion and indirectly inhibits autophagy, an event which in turn causes accumulation of misfolded proteins, ER stress and cell death through apoptosis. Despite our model is well established, FAM46C mechanistic mode of action and physiological function still require elucidation, as well as its involvement in the onset of other cancers.

Given the vast interactome of FAM46C, we hypothesize that the protein might regulate - and/or be regulated by - a vast number of intracellular pathways.

By defining the intracellular networks which regulate FAM46C expression/function and those that are being regulated by FAM46C, we plan to better define its mode of action and ultimately discover novel druggable candidates important for implementing patient therapy.

Backed by strong evidence that the FAM46C complex might function as an oncosuppressor also in breast cancer (BC), we extended our studies outside of the MM environment and specifically in breast malignancies.

Aims

To ultimately identify novel druggable targets for cancer therapy implementation, our general goal was to define, through genetic and biochemical approaches, the intracellular networks involved in FAM46C oncosuppresor complex functionality.

Specifically our research plan was divided in two areas: AREA 1, focusing on better defining the general role of FAM46C and AREA 2, focusing on the effects of FAM46C expression in BC.

Our specific aims for the two AREAS were the following:

-1 - Define the intracellular networks associated with FAM46C functionality.

-2 - Dissect the oncosuppressor features of FAM46C in breast cancer.

-3 - Define FAM46C function in breast cancer.

Experimental design

To define which intracellular networks are regulated by FAM46C we focused on two main tasks:

-Task 1.1) Validation and functional characterization of the FAM46C/BCCIP interaction

and

-Task 1.2) Definition of the pathways involved in regulating FAM46C expression.

For the first task we selected BCCIP because it is a protein involved in vesicle trafficking dynamics and tumour onset, and because strong evidence in our hands indicated that it physically interacted with FAM46C. The interaction was validated through biochemical approaches and its functional relevance was analysed by modulating its protein expression and assessing selected FAM46C-induced phenotypes.
For the second task, we originally planned to define FAM46C genetic interactions by performing a genome-wide sh-RNA screen. However, having encountered practical problems, we opted for an alternative approach based on data mining of previous MM RNA-seq data produced in the lab. We found a strong correlation between FAM46C expression and IFN-α and -γ signatures, suggesting FAM46C to be involved in such type of intracellular responses. Given that IFN signalling pathways are connected to antiviral responses and driven by the evidence that wt FAM46C lentiviruses were always produced with less efficiency compared to those expressing the D90G mutant, a loss-of-function variant of FAM46C, we tested if FAM46C could be involved in regulating viral particle production and explored the idea of envisaging FAM46C as an antiviral protein.
For what concerns dissection of FAM46C features in BC we focused on: 1) verifying that FAM46C was indeed a tumour suppressor also in BC by performing proliferation, migration and invasion assays (taks 2.1) and 2) defining the mode of action of FAM46C in BC (task 2.2.), by testing all the well-established FAM46C-induced phenotypes, namely: induction of apoptosis, protein aggregate accumulation and autophagic impairment. Moreover, given that FAM46C functionality in MM requires its ER localization thanks to interaction with FNDC3A, we also assessed the existence of this interaction through co-immunoprecipitation.

Results

For what concerns task 1.1, physical interaction between FAM46C and BCCIP was confirmed and also validated with FAM46C mutant allele D90G, by both immunoprecipitation, immunofluorescence and pulldown experiments. Intriguingly we found that BCCIP binds the FAM46C-D90G mutant allele with more affinity compared to wt FAM46C. This differential binding is in agreement with our working hypothesis in which this interaction is expected to negatively regulate FAM46C functionality. In this direction we tested if modulation of BCCIP levels could affect FAM46C capability to inhibit autophagy and indeed found that downmodulation of BCCIP in FAM46C-expressing cells favoured autophagic inhibition.
Concerning task 1.2. we found that FAM46C is indeed an interferon-stimulated gene and that the expression of wild-type FAM46C, but not of its most frequently found mutant variants, inhibits HIV-1 lentiviral particle production through a mechanism that relies on FAM46C-induced deregulation of autophagy.
Concerning FAM46C involvement in BC, we actually demonstrated that FAM46C has clear oncosuppressor features as its expression correlates with decreased proliferation, migration and invasion of BC cells. Mechanistically FAM46C expression induced apoptosis, protein aggregate accumulation and triggered autophagic impairment, suggesting that the same mode of action carried out by FAM46C in MM is actually functioning also in BC. Accordingly, we have demonstrated that a FAM46C/FNDC3A complex is formed also in BC cells as co-immunoprecipitation experiments showed that FAM46C actually interacts with FNCD3A.

Conclusions

Overall, so far, we were able to:

1) Better define the pathways regulating FAM46C expression, as we demonstrated that FAM46C is expressed downstream of IFN-α and -γ signalling.

2) Describe a novel role associated with FAM46C, namely inhibition of viral particle production.

3) Confirm the interaction between FAM46C and BCCIP and demonstrate that BCCIP is possibly an inhibitor of FAM46C.

4) Demonstrate that FAM46C has tumour suppressor features also in BC, where it seems to regulate, at least in part, the same pathways regulated in MM.

These findings, will set the foundations for reaching our ultimate goal: defining novel broad-cancer or cancer-specific targetable pathways/genes.

Luca Mazzarella

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Intra-tubular damage is targeted by maytansinoids and rescued by NF1: Revisiting mechanism and biomarkers of an established ADC payload

Background and hypothesis

There is great interest in the identification of biomarkers to guide development of antibody-drug conjugates (ADC). We previously showed that loss of Neurofibromatosis 1 (NF1), a gene frequently mutated across cancers, enhances the activity of DM1, the maytansinoid payload of T-DM1, through a novel function in regulating microtubule (MT) dynamics. Maytansinoids are puzzlingly more effective in cells (in the nanomolar range) vs in vitro (in the micromolar range). Since maytansinoids bind at the interface between tubulin dimers, they are thought to only bind soluble tubulin dimers or MT ends, which would suggest very few binding sites available for pharmacological interaction in vivo, at odds with data.

Aims

We investigated the interaction of DM1 with NF1 and MTs, with the aim of characterizing a potential biomarker for ADC use in the clinics

Experimental design

To measure in vivo MT dynamics, we transiently transfected the MT end-binding protein EB3-GFP and reconstructed MT trajectories by live-cell imaging. The effect of DM1 and NF1 on tubulin polymerization was characterised by turbidity-based tubulin polymerization assays. To follow the dynamics of individual microtubules, we applied Total Internal Reflection (TIRF) microscopy on glass-immobilized MTs.

Results

Upon DM1 treatment, KO cells showed a highly significant reduction in MT speed, demonstrating a direct role for NF1 on MT dynamics in cells. In recombinant NF1 greatly accelerated polymerization, and completely rescued DM1-induced inhibition. Visual inspection of fluorescent MTs showed that NF1 induced significant MT bundling, a defining feature of many MT-associated proteins, which generates signal indistinguishable from true MT polymerization in turbidity assays. As expected, polymerization in the presence of NF1 led to a significant increase in MT dynamics (elongation speed, rescue and catastrophe rate). Expectedly, DM1 led to significant reduction in the fraction of elongating MTs and speed, but these defects were completely or partially rescued by NF1. Importantly, DM1 did not only lead to MT shortening (as proposed by the current model), but also to clear and frequent MT fracturing, indicating that the drug is not only engaging MT ends but also intra-tubular binding sites. This is consistent with recent models of MT formation which incorporate the frequent presence of areas of discontinuity or damage induced by mechanical stress, exposing intra-tubular DM1 binding sites. Interestingly, adding NF1 to DM1-treated MTs generated areas of de novo intra-tubular tubulin insertion, coincident with damaged sites, suggesting an entirely novel role for NF1 in MT repair.

Conclusions

In conclusion, we provide evidence for a model in which maytansinoids bind not only to soluble tubulin

dimers and MT ends, but also to intra-tubular damaged sites. Thus, the number of binding sites in cells would be proportional to MT damage, suggesting a mechanism for differential efficacy across tumor types and a potential avenue for combinatorial drug development.

Alessio Menga

Università degli Studi di Torino, Torino

Dissecting mitochondrial lysine and tryptophan metabolism to target metabolic symbiosis in lung adenocarcinoma.

Background and hypothesis

This research explores a novel avenue in lung adenocarcinoma (LUAD) by investigating the contribution of alternative amino acids, namely tryptophan and lysine, to cancer cell behavior. Traditionally, research has focused on the role of glucose and glutamine metabolism in LUAD. This study proposes a distinct metabolic pathway involving DHTKD1, a crucial mitochondrial enzyme for tryptophan and lysine catabolism, which may significantly impact tumor progression and immune evasion in LUAD by modulating for instance the glutarylome (histones, mitochondrial genes). Targeting DHTKD1 presents a potential therapeutic strategy to disrupt this novel pathway and potentially inhibit the aggressive phenotype of LUAD cells.

Aims

This study has three key aims:

1. Uncover how DHTKD1 affects LUAD aggressiveness and drug response.
2. Explore DHTKD1's role in creating an immune-tolerant tumor environment.
3. Evaluate DHTKD1 as a potential target linked to LUAD severity and immune escape.

Experimental design

We used a combination of cell studies (in vitro and in vivo) and advanced genetic, metabolic, and bioinformatic techniques to understand how silencing DHTKD1 in lung cancer cells affects their metabolism, immune response, and overall behavior. We also analyzed human tumor samples to see if higher DHTKD1 levels correlated with more aggressive cancer.

Results

Silencing DHTKD1 in cancer cells resulted in unanticipated metabolic changes: increased oxidative metabolism, dependence on glucose, and elevated ROS with hypoxia sensitivity. Notably, decreased mitochondrial tryptophan catabolism led to a shift towards the serotonin pathway, with accumulation and secretion of 5-HIAA. In vivo studies with silenced cells showed significant tumor reduction, improved blood vessel formation, decreased collagen and hypoxia, compared to controls. Analysis revealed a rise in tumor-infiltrating CD8+ T cells, likely due to reduced collagen deposition (known to hinder CD8+ T cell function). Interestingly, silenced tumors also showed increased eosinophils, known to orchestrate cancer rejection by normalizing tumor vessels and enhancing infiltration of CD8(+) T cells. Human tissue analysis confirmed a positive correlation between high DHTKD1 levels and poor prognosis (tumor grade). Additionally, "DHTKD1-high" tumors displayed increased collagen remodeling, suppressed immune pathways, and more T regulatory cells (potentially linked to immunotherapy resistance). Conversely, tumors with lower DHTKD1 had higher numbers of CD8+ T cells and eosinophils, suggesting DHTKD1's role in regulating the anti-tumor

immune response.

Conclusions

High DHTKD1 levels in LUAD link to poor prognosis, suggesting its use as a biomarker. Knocking down DHTKD1 in mouse models shrank tumors, improved blood vessel formation, and reduced hypoxia. Importantly, it also boosted infiltration of immune cells, particularly CD8+ T cells and eosinophils. The study proposes a potential role for the accumulated tryptophan metabolite 5-HIAA in eosinophils recruitment via the 5-HIAA-GPR35 ligand-receptor system, but further research is needed to confirm the mechanism.

Francesco Neri

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Origin and evolution of cancer-associated intestinal epigenetic drift

Background and hypothesis

The accumulation of age-related DNA methylation (DNAm) alterations is linked to cancer development. However, the mechanistic origins and functional implications of age-dependent DNAm changes in cancer initiation remain poorly understood. Recent findings reveal a tissue-specific DNAm drift in intestinal stem cells (ISCs) during aging, highly enriched in colon cancer, involving promoter hypermethylation of specific genes. Unlike the CpG island methylator phenotype (CIMP), this DNAm drift is pervasive across colon cancer types. Our hypothesis, supported by previous research, is that ISCs with high levels of this DNAm drift serve as the cell of origin of intestinal cancers.

Aims

We want to study these aging- and colon cancer-associated DNAm alterations to understand how they are originated and their impact in the initial phases of cellular transformation

Experimental design

We successfully established protocols to isolate and study intestinal stem cells from old mice that have high level of this specific DNAm drift. We employ intestinal organoid models from mice, healthy human samples, cancer human samples and IPS-derived cultures.

Results

Our results elucidate the origin of these aging- and colon cancer-associated DNAm alterations.

Conclusions

By deciphering the underlying mechanisms and pathways involved, we anticipate insights into cancer prevention strategies and diagnostic tools, while also providing new targets for the development of therapeutic approaches.

Silvia Ravera

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Investigating Biochemical and Molecular Markers Responsible for Early Aging in Childhood Cancer Survivor.

Background and hypothesis

Although survival rates of Childhood Cancer Patients have improved over the past four decades, Childhood Cancer Survivors (CCS) clearly show the risk of possible long-term clinical complications related to chemo/radiotherapy and consistent with early aging. However, the cellular/molecular basis of all described symptoms and signs remain missing.

Among the alterations involved in the aging process, mitochondrial metabolism and the consequent increment of oxidative stress production play a pivotal role.

Aims

Thus, using mononuclear cells (MNCs) isolated from CCS peripheral blood, the oxidative phosphorylation (OxPhos) efficiency, oxidative stress/ antioxidant defenses balance, and mitochondrial dynamics markers were evaluated, comparing the results to those obtained on MNCs isolated from age-matched healthy donor and elderly subjects.

Experimental design

Luminometric, oximetric, spectrophotometric, and proteomic analyses were performed on 196 CCS samples aged between 5 and 20 years, comparing the results with those obtained on MNCs of 154 healthy subjects aged between 5 and 106 years old.

Results

Data show that the CCS OxPhos efficiency decreased compared to the healthy age-matched samples but was similar to that observed in the elderly subjects. In addition, an increment of oxidative stress with respect to the healthy age-matched population was observed, despite antioxidant defense activation. In addition, by applying a mathematical model predicting the age based on glucose metabolism, CCS displayed a biological age significantly increased by decades compared to the chronological age. CCS but not healthy age-matched and elderly subjects showed a 5-fold downregulation of CLUH, PGC1-α, and SIRT6 gene expression, suggesting that the altered expression of these genes could not be linked with physiological aging. In addition, CCS MNCs showed an unbalance between mitochondrial fusion and fission and altered mitophagy and autophagy pathways.

Conclusions

In conclusion, this study identified some biochemical and molecular alterations possibly contributing to the pathophysiology of aging and metabolic deficiencies in CCS.

Mariangela Russo

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Leveraging DNA Damage Response to prevent secondary resistance to MAPK pathway inhibition in CRC.

Background and hypothesis

Development of secondary resistance represents the major obstacle to tumor eradication. While in some cases, cells harboring mechanism of resistance might already be present in tumor lesions before drug administration, in a fraction of patients surviving drug tolerant persister (DTP) cells play a key role in the failure of clinical treatment. DTPs are able to survive to the lethal effect of anti-cancer agents through transient non-genetic mechanisms of drug tolerance. However, prolonged exposure to drug-induced hostile environment induces the activation of a stress-response, named adaptive mutability, characterized by a switch to low fidelity DNA replication process, in the presence of increased DNA damage and impaired mismatch repair (MMR) and homologous recombination (HR) proficiency. This in turn leads to increased genetic instability and temporary increase of their mutation rate.

The evidence that targeted therapies alter the proficiency of DNA damage recognition and repair (DDR) might unveil new therapeutic targets and/or combinatorial strategies able to prevent the onset of resistance, and therefore disease recurrence.

Aims

This project aims to identify and intercept the key players of adaptive mutability to unveil and restrain the cellular mechanisms driving drug-induced mutagenesis during therapeutic treatment in colorectal cancer (CRC).

Experimental design

To pursue our aims, we selected a panel of CRC cell lines with known sensitivity to targeted therapies commonly used in the clinical setting. Pharmacological and CRISPR-CAS9-based genetic screenings were exploited to identify DDR players involved in adaptive mutability and to characterize their effect on development of secondary resistance to targeted therapies. Fluorescent-based and functional assays were used to dissect the mechanism of action of combinatorial inhibition of DDR and MAPK pathways in CRC.

Results

Taking advantage from different DDR inhibitors (e.g. ATR, ATM, WEE1, DNA-PK, REV1) currently in clinical development, we assessed their effect on CRC cells viability in combinatorial drug screenings with cell-specific oncogenic inhibitors. We found that all DDR inhibitors enhanced the cytotoxic effect of targeted therapies across different CRC models. Time To Progression assay unveiled that DDR inhibitors were effective in delaying or, in some cases, even preventing the development of resistance in all CRC models tested, with DNA-PK, REV1 and WEE1 being the most powerful in synergizing the MAPK pathway inhibition. Notably, the addition to targeted therapy of selected DDR inhibitors significantly reduced the percentage of surviving DTPs, thus impairing the reservoir of mechanisms of resistance. This was, at least in part, due to a

significant increase of ROS levels and DNA damage.
In parallel we exploited a custom CRISPR DDR library to identify genes modulating adaptive mutability in cancer cells. WiDr CRC CAS9 cells infected with CRISPR DDR library, after 5 days of puromycin selection, were grown in absence or presence of oncogenic inhibitors till development of secondary resistance. A negative selection bioinformatic analysis of sgRNA absent in the resistant cells, will allow the identification of genes whose knock-out (KO) impaired the development of drug resistance to targeted therapy, thus representing new potential therapeutic targets.

Conclusions

Leveraging the knowledge that cancer cells impair MMR and HR under therapeutic stress, we unveiled novel possible targets and therapeutic strategies, based on the concomitant inhibition of MAPK and DDR pathways, able to interfere with clonal evolution, thus preventing the development of secondary resistance and prolonging the efficacy of clinically approved targeted therapies in CRC.

Alessandro Sessa

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Engineered epigenetic silencers repress glioblastoma growth

Background and hypothesis

Current therapies remain unsatisfactory in preventing the recurrence of glioblastoma multiforme (GBM), which leads to poor patient survival. Major evidence indicates that cancer cells with tumor-initiating potential infiltrate the parenchymal tissue and become resistant to adjuvant treatments, thereby supporting the recurrence of the disease.

A treatment based on the administration of epigenetic editors in vivo, e.g. post-surgery brain, can extinguish the GBM resistant cells and minimize the risk of tumor reappearance, without risk for the surrounding (healthy) cells.

Aims

To define the role of a new class of epigenetic silencers in coordinating the anti-tumor activity (1), their effective functionality in preventing tumor growth (2), and their safety (3).

Experimental design

By rational engineering of oncogenic transcription factors through the rational assembling with domains from chromatin and transcriptional epigenetic repressors, we generated a family of synthetic epigenetic silencer factors (ESFs). The modified transcription factors include SOX2 and TEAD1 which have of the key in GBM. Through the addition of the KRAB and DNA methyltransferase 3A/L catalytic domains, we generated synthetic repressors named SOX2 epigenetic silencer (SES) and TEAD1 epigenetic Silencer (TES). Both ESFs were tested in in vitro and in vivo models of GBM as well as their molecular activity, transcriptional output and anti-tumoral outcome.

Results

SES and TES inhibit both glioma cell lines and patient-derived cancer stem cells in vitro and in vivo. Their expression, through local viral delivery in mouse xenografts, induces strong regression of human tumors and survival rescue. SES produces a significant silencing of a large fraction of the SOX2 transcriptional network, achieving high levels of efficacy in repressing aggressive brain tumors. TES induced the selective and robust transcriptional silencing of the YAP/TAZ validated targets, through epigenetic inhibition as assessed by RNA-seq. Conversely, they are not harmful to neurons and glia, representing e relevant option of GBM therapy.

Conclusions

Collectively, this rational protein engineering approach produced a significant silencing of a large part of the tumorigenic transcriptional network, achieving high levels of efficacy and safety for the treatment of aggressive brain tumors. Given its wide applicability to other oncogenic TFs and the efficiency of targeting cancer cells by viral transduction, this approach offers an innovative strategy to build antitumor molecular tools effective against glioblastoma and other deadly cancers.

Maura Sonego

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Targeting the deubiquitinase USP1 to improve therapy response in ovarian cancer

Background and hypothesis

Epithelial Ovarian Cancer (EOC) treatment has been revolutionized by the introduction of PARP inhibitor (PARPi) maintenance therapy in platinum (PT) sensitive patients. This was due to the proved synthetic lethality between PARPi and the deficiency in the Homologous Recombination (HRD) DNA repair pathway. Accordingly, PARPi are extremely active in patients with BRCA1/2 mutated EOC and in a subgroup of BRCA WT ones. However, resistance to PT/PARPi has been clinically described with consequent disease relapse and discouraging prognosis. PARPi exert their cytotoxic effect acting mainly on PARP1 by both trapping PARP1 on the damaged DNA and restraining its poly-ADP-ribosylation activity (PARylation). How PARP1 is recruited and trapped at the site of DNA damage and how resistance to PARPi could be overcome is still matter of investigation. The USP1 deubiquitinase is a prominent oncoprotein in EOC involved in both DNA repair and stem cell maintenance. Here we studied the role of USP1 in the regulation of PARP1 activity and PARPi response in EOC.

Aims

Our main goals were to dissect the molecular mechanisms by which USP1 participates in the response to DNA damage repair, in particular during replicative stress and replication fork stalling. Moreover, we have defined the role of USP1 in the regulation of PARP1 activity and PARPi response in EOC. With the clarification of these mechanisms we will improve the knowledge on PT/PARPi-resistance and possibly impact on the management of EOC patients.

Experimental design

Genetic (CRISPR/Cas9 technology) and pharmacological (USP1 and PARP inhibitors) approaches were used in dose-response curves to assess cell viability of PT/PARPi sensitive and resistant EOC cells, in primary cultures derived from the ascites EOC patients and in vivo in EOC PDX model. Co-immunoprecipitation (CoIP), Ubiquitin pull down, DNA Fiber, Comet, iPOND and Proximity Ligation Assays (PLA) were employed to clarify the molecular mechanisms by which USP1 regulates PARPi response.

Results

By investigating the role of the deubiquitinase USP1 in the response to PARPi treatment, we found that USP1 inhibition sensitizes ovarian cancer cells to PARPi activity irrespective of their HR-status. These biological data had clear molecular explanations. We proved that PARP1 is a substrate of USP1 and that USP1 binds PARP1 to remove its K63-linked poly-ubiquitination. PARP1 poly-ubiquitination dynamically regulates its chromatin trapping and catalytic activity, therefore modulating cells sensitivity to PARPi. Accordingly, in both PT/PARPi sensitive and resistant cells, combined USP1/PARP1 inhibition enhances replicative stress, DNA damage and cell death.

Conclusions

Our work, by studying the role of PARP1 ubiquitination in ovarian cancer, describes a relevant biological

interaction between USP1 and PARP1. In this scenario, we propose that USP1/PARP1 axis represent a novel druggable target to be explored as promising therapeutic choice not only for sensitive but, more importantly, for chemoresistant ovarian cancer patients

Paola Storti

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Single cell transcriptomics of the bone microenvironment cells reveals altered features in multiple myeloma patients compared to pre-malignant monoclonal gammopathies

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Background and hypothesis

Multiple myeloma (MM) is a malignant plasma cell dyscrasia that can be preceded by monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM). How the bone microenvironment (BME) changes and contributes to tumoral progression in MM remains unresolved. Published data on BME are mainly produced in vitro and few data are on MGUS/SMM patients. Up to date, no single-cell RNA sequencing database of the BME cells has been described in MM.

Aims

The aim of this project was to characterize for the first time at single cell level, the BME in patients with newly diagnosed MM (MMD) and MGUS/SMM to identify alterations involved in tumoral progression

Experimental design

From 16 bone biopsies of MMD, MGUS and SMM patients, we depleted CD235a+, CD45+, CD31+, and CD138+ cells to enrich the rare BME non-hemopoietic cells. The CD45-CD31-CD235a-CD138- cells were analyzed by scRNAseq. Data were generated on Chromium 10X Genomics. Cellranger and Seurat pipeline in R software has been used. Cell identities were assigned by manual curation. The trajectory analysis has been made by Monocle3. Decouple R, GSVA and FindMarkers were used to compare pathways of the samples throughout the different conditions.

Results

A total of 44.163 cells were profiled. We identified 16 BME cell type clusters: 10 mesenchymal stromal cells (MSCs) clusters and 4 osteoblasts (OBs) clusters and 2 unknown clusters. Pseudotime analyses highlighted, for the first time in MM setting, several and complex trajectories of differentiation from more immature cell clusters to mature OB cluster, confirming the ability of the dataset to snapshot the in vivo complexity of patient's bone microenvironment. Subsequently, we have split the database in the 3 groups of analysis: the distribution of the proportion of the different cell types between MGUS/SMM/MMD was altered and we highlighted an increasing reduction and destruction of the MSC route of differentiations from MGUS samples to MMD. Moreover, MGUS/SMM sample showed a peculiar expression dynamic of early osteoblasts differentiation markers as DLX5, RUNX2 and their downstream targets (IBSP, SPP1, BGLAP) in pre-osteoblastic cell type with a consequent decrease of their expression on more mature cells and an upregulation of OB markers. This dynamic in MMD samples is completely altered and in the differentiated cells, it is blocked in the higher RUNX2 expression with a consequent lack of expression of OB markers.
Finally, we have highlighted several pathways and biological processes (BPs) altered in the progression to MM: MGUS sample showed upregulation of pro-osteogenic and immune activator pathways, MMD samples BPs involved in B-cell homeostasis and suppression of OBs. The SMM samples showed an upregulation of

BPs involved both in the promotion or the inhibition of osteoblastogenesis and already an upregulation of B cell-supporting process compared to MGUS, likely underling the attempt of SMM patients to contrast the establishment of altered BME, characteristic of MM.

Conclusions

Our approach is able to dissect the complex organization of the BME and to highlight at single cells level the alterations of the BME in patients with MM compared to MGUS and SMM.

Simone Tamburri

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Defining the role of PCGF3-containing Polycomb Repressive Complex 1 in Synovial Sarcoma

Background and hypothesis

Human synovial sarcoma (SS) is a rare and aggressive soft tissue tumor, primarily affecting younger individuals, posing significant challenges in its management due to its high metastatic potential. The driving force behind SS is a specific chromosomal translocation, t(X;18)(p11.2;q11.2), resulting in the fusion of the SS18 gene on chromosome 18 with either SSX1, SSX2, or, less frequently, SSX4 on chromosome X. This fusion generates the hallmark SS18-SSX chimeric oncoprotein which replaces the wild type SS18 subunit and evicts the tumor suppressor SMARCB1 (also known as BAF47 or hSNF5) which is one the main component of the BAF complex, a chromatin remodeling complex, disrupting its normal genomic activity and contributing to the oncogenic process in SS. It has been demonstrated that 34 aminoacids in the SSX-C terminus are essential for the binding of the SS18-SSX on the chromatin and exhibit preferential binding to H2AK119ub-modified nucleosomes, a histone modification deposited by the Polycomb Repressive complex 1 (PRC1). Therefore, the oncoprotein shifted the BAF complex activity from the active genomic regions to Polycomb-repressed domains, contributing to the oncogenic process in SS.

The majority of H2Aub1 is diffusely deposited at low levels across the genome by the specific activity of PRC1.3/5, one of the sub-complexes or PRC1. Moreover, PCGF3, a core component of the subcomplex PRC1.3, was identified as the strongest synthetic lethality hit in a cohort of SS cell lines through a meta-analysis in the Dependency Map (DepMap) portal.

Aims

This project aims to uncover the mechanisms that connect Polycomb repression to deposition of H2Aub1 in oncogenic conditions in which H2Aub1 homeostasis is disrupted such as the aberrant reading of H2Aub1 is represented by the modified nucleosomes by the SS18-SSX oncogenic fusion protein that drives Synovial Sarcoma (SS) development.

Experimental design

Since general inhibition of PRC1 activity affects global cells viability, targeting specific PRC1 subcomplexes, such as the PRC1.3 subcomplex, could become an attractive strategy. However, we still lack knowledge about the role played by each specific sub-complexes in different cellular contexts. Similarly, we lack understanding about the molecular details that govern and distinguish PRC1 sub-complexes. Despite their composition have been well characterized, very little is known about the biochemical features that govern their assembly, sustain their targeting to chromatin and enzymatic activity. This information is essential to comprehend their activity and become crucial for designing new therapeutic strategies.

We aim to leverage the obtained biochemical insights to uncover, at a genome-wide level, the mechanisms linking PRC1 activity with oncogenic H2Aub1 in the context of SS18-SSX fusion expression (SS).

Results

To characterize the mechanisms underlying PCGF3 synthetic lethality, we employed two complementary approaches, the CRISPR/Cas9 technology and dTAG system to deplete PCGF3. We demonstrated that loss of PCGF3/5 expression in SS cell lines globally displace SS18-SSX chromatin association, compromising global chromatin acetylation SS cells viability. Loss of PRC1.3/5 activity in two independent SS cell lines indeed resulted in a global reduction of H2AK119ub levels, suggesting a molecular circuit that connects SS18-SSX activity with PRC1.3/5-dependent H2Aub1 diffused genomic deposition. However, despite the H2AK119ub serves as a binding surface for SS18-SSX, our results demonstrated that it was not sufficient to fully elucidate the molecular mechanisms underlying PCGF3 lethality. Our results clearly showed that other mechanisms independent from the PRC1 are linked to the role played by PCGF3 in maintain the SS survival. Indeed, PCGF3 depletion in PCGF3-sensitive cell lines induced an increase of p53 and p21 protein levels. On the other hand, insensitive cell lines displayed high basal levels of p53 and p21 compared to the sensitive cell lines, suggesting a potential mechanism bypassing PCGF3 loss for these cells.

Conclusions

Unraveling this molecular mechanism holds the key for a deeper understanding of the molecular pathways dictating SS proliferation, with significant clinical implications.

Marcella Tazzari

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Unveiling the function of tertiary lymphoid structures and associated CXCL13 to enhance immunotherapy in solid tumors

Background and hypothesis

The response to immunotherapy (IT), particularly immune checkpoint inhibition (ICI), demonstrates enduring effects only in a subset of patients. Tumors with an inflamed tumor microenvironment (TME), characterized by tertiary lymphoid structures (TLSs), correlate strongly with positive IT outcomes. However, real-world data indicate that even tumors housing TLSs may progress. Understanding the reciprocal interaction of TLSs with other immune determinants and cancer cells, tailored to individual tumor types, is crucial for developing optimized treatment strategies.

Aims

This project aims to reclassify intra-tumoral B cell enrichment alongside other immune cell subsets and elucidate the impact of CXCL13 on the antitumor immune response.

Experimental design

Our study includes retrospective and prospective cohorts of melanoma patients receiving ICI in metastatic, adjuvant, neoadjuvant settings, and Head and Neck Squamous Cell Carcinoma (HNSCC) samples from patients progressing or not to ICI. A cohort of pre and post-therapy lesions from dendritic cell-vaccinated melanoma patients was also analyzed. Immunohistochemistry (IHC) was used to determine the abundance and reciprocal distribution of CD3, CD20, CD21, CD8, and CD163. Additionally, single-cell RNA sequencing was conducted on IT-resistant (ITr) lesions, and an in-house sequential IHC approach was developed to validate up to 8 markers on the same slides. Cancer cell lines from both pathologies were screened for CXCL13/CXCR5 and applied in in vitro assays.

Results

Three categories were defined: low B cell (Cat.1), medium/high presence of B cells but not organized (Cat.2), and TLS-organized B cells (Cat.3). Notably, Cat.3 does not always translate into higher B cell abundance, but rather into compartmentalization of B cells in specific nests. This B cell scoring system was combined with the annotation of T cell or macrophage prevalence. We observed a spectrum of B cell infiltration in lesions from both melanoma and HNSCC patients. Notably, Cat. 2 and 3 were observed across patients with disease progression, often accompanied by a predominance of macrophages over T cells. Single-cell RNA transcriptomic analysis was conducted on 4 ITr melanoma samples. While CXCL13 has often been linked with TLS promotion and IT response, our data demonstrate the presence of CXCL13-producing CD8+ T cells in ITr lesions. Using ProjecTILS, we highlighted the presence of two major CXCL13-producing CD8+ T cells: progenitor exhausted PD1+TCF1+T cells (Tpex) and exhausted PD1highTIM3highT cells (Tex). In vitro modeling showed that, while initially, tumor-reactive CXCL13+ T cells produce effector molecules (IL-2, IFN-γ, TNF-α), they become hypofunctional and replicate high inhibitory receptor expression upon chronic

stimulation. We also found CXCR5 expression, mainly intracellularly, in both HNSCC and melanoma cell lines.

Conclusions

Our data support two different, non-exclusive, biological trajectories aligned with our original hypotheses: i) TLSs may be dampened in their anti-tumor effect by outnumbered macrophages; ii) CXCL13+ CD8+ T cells may exist irrespective of B cells, as a consequence of antigen-driven exhaustion. We are integrating macrophage phenotyping into the existing TLS signature to advance their use in patient stratification, replacing the TLS signature alone as a measure of IT responsiveness. Additionally, we are investigating Tex-derived CXCL13 generation within the TME and assessing the intra-tumoral effect of CXCL13.

Toma Tebaldi

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Interpreting single-cell messages in normal and aberrant hematopoiesis with the Cell Marker Accordion

Background and hypothesis

Single-cell technologies offer a unique opportunity to explore cellular heterogeneity in hematopoiesis, reveal malignant hematopoietic cells with clinically significant features and measure gene signatures linked to pathological pathways. However, reliable identification of cell types is a crucial bottleneck in single-cell analysis. Available databases contain dissimilar nomenclature and non-concurrent marker sets, leading to inconsistent annotations and poor interpretability. Furthermore, current tools focus mostly on physiological cell types, lacking extensive applicability in disease.

Aims

We developed the Cell Marker Accordion, a user-friendly platform for the automatic annotation and biological interpretation of single-cell populations based on consistency-weighted markers.

Experimental design

We validated our approach on peripheral blood and bone marrow single-cell datasets, using surface markers and expert-based annotation as the ground truth. In all cases, we significantly improved the accuracy in identifying cell types with respect to any single source database.

Results

The Cell Marker Accordion can identify disease-critical cells and pathological processes, extracting potential biomarkers in a wide variety of contexts in human and murine single-cell datasets. It characterizes leukemia stem cell subtypes, including therapy-resistant cells in acute myeloid leukemia patients; it identifies malignant plasma cells in multiple myeloma samples; it dissects cell type alterations in splicing factor-mutant cells from myelodysplastic syndrome patients; it discovers activation of innate immunity pathways in bone marrow from mice treated with METTL3 inhibitors.

Conclusions

The breadth of these applications elevates the Cell Marker Accordion as a flexible, faithful and standardized tool to annotate and interpret hematopoietic populations in single-cell datasets focused on the study of hematopoietic development and disease.

PARTICIPANTS

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Engineered Extracellular Vesicles for personalized leukemia therapy

Background

During the last few years, the treatment of cancer patients has been revolutionized by modern oncology, with a deeper understanding of cancer cells at the molecular level moving forward to personalized medicine. The classic broad anti-cancer drugs are now often combined with more tailor-made treatments (e.g.immunotherapy), to target each cancer type more precisely and with increased efficacy. Despite current advances, there is still a need for more specific targeted therapies, a long-sought goal to target tumors for complete eradication.

Hypothesis

The ideal treatment effectively and specifically targets cancer cells, limiting possible side effects. Extracellular vesicles (EVs) are promising carriers of anti-cancer drugs to achieve this goal. However, two main problems are currently limiting the use of EVs for therapy: low cargo delivery of EVs into target cells and low enrichment of the therapeutic protein of interest into EVs. Here, we hypothesize that, if ad hoc engineered, EVs can be loaded with any cargo protein and re-directed against any specific target cell.

Aims

This project aims at generating a platform of engineered EVs (eEVs) with multiple features to specifically target different cancer cells. Acute lymphocytic leukemia (ALL) and chronic lymphocytic leukemia (CLL) are selected in this proposal as proof-of-concept model to test our hypothesis. ALL and CLL have been chosen due to their lymphocytic nature, their main localization in the bloodstream, and the easy access to patient samples. To monitor EV cargo delivery into target cells, we recently established a novel EV fusion assay. eEVs selected by fusion assay to be able to enter ALL and CLL will then be loaded with CRISPR/Cas9 ribonucleoproteins that carry guideRNAs to target leukemia-specific genomic alterations.

Experimental design

Engineered EVs will be designed with envelopes from different lymphotropic viruses (e.g. EBV and HIV-1) in combination with antibodies specific to antigens expressed by leukemia cells and with fusogenic molecules, to fuse efficiently to the target cells. For the cargo delivery, we will use a tunable self-cleavage-protease system in which Cas9 is fused with CD63 that confers enrichment into EVs, and with a protease capable of cleavage in cis, allowing cargo release into the target cells. To better control the Cas9 delivery, the system can be easily fine-tuned by a specific protease inhibitor.

Expected results

We will generate a list of eEVs that will cover specificity for ALL and CLL from different patients. eEVs will first be challenged against in vitro and ex vivo models of leukemia to improve EVs delivery and anti-tumor effector function. Additionally, the most effective anti-ALL eEVs will be validated in vivo in a ALL patient-derived xenograft (PDX) model. Once established, the platform will consist of a combination of eEVs with different targeting/fusogenic machineries and Cas9-gRNAs, capable of targeting different genomic alterations in a tumor and patient-specific manner.

Impact on cancer

This novel therapeutic approach is going to revolutionize current cancer treatments. eEVs will be a new class of extremely flexible drugs that can be adapted for each type of malignancy and to each patient, providing a completely personalized and precise therapeutic option to replace or be combined with already available treatments.

Francesco Boccalatte

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Epigenetic characterization of pediatric T cell leukemias

Background

T cell Acute Lymphoblastic Leukemia (T-ALL) is an aggressive blood malignancy with a high incidence in the pediatric age. Despite major improvements in clinical management and outcome of T-ALL, almost 25% of patients fail frontline therapy or experience early relapses, with a negative impact on their survival. This is particularly evident in specific disease subgroups, suggesting an unrecognized biological complexity that might contribute to drug resistance. So far, many efforts have been invested in the characterization of T-ALL at the genetic level, focusing on the mutational cause of relapse, but very few studies have explored the epigenetic determinants of leukemia based on chromatin conformation.

Thanks to the advance of state-of-the-art sequencing techniques, it is now evident that the 3D chromatin conformation is an important layer of control of cell properties and functions. Aberrant organization of chromatin topology appears particularly relevant in the context of cancer, where atypical looping events can cause long-range interaction to occur between enhancer elements and the promoters of proto-oncogenes, thus favoring cell growth.

Hypothesis

The main hypothesis of this study is that genome-wide epigenetic changes at the 3D chromatin level are key determinants of cellular transformation and sustain drug resistance in T cell leukemia. Particular attention will be given to the rewiring of enhancer elements, which allow for long-distance control of genetic loci.

Aims

Aim 1: Genome-wide alterations of chromatin topology in acute T cell leukemia

Aim 2: Identification and validation of enhancer hubs in leukemia

Aim 3: Patient-derived models expose epigenetic resistance to chemotherapy

Experimental design

With the current proposal, I aim to demonstrate that the global reorganization of 3D chromatin architecture, including TAD structure modifications and enhancer rewiring, can both initiate cellular transformation and promote drug resistance. I will investigate this hypothesis in the context of T-ALL, where I will follow the evolution of 3D reorganization at diagnosis and relapse, validating new elements that control oncogene activity and their adaptation upon treatment. Particular attention will be given to enhancer hubs, under the hypothesis that hyper-connected enhancer elements may represent master regulators of leukemia development and hold therapeutic significance. Epigenetic elements will be validated by synergistic targeting both in vitro and in vivo using dedicated models of relapse, in order to find vulnerability elements for T-ALL treatment.

Expected results

We expect that this study will clarify the role of 3D chromatin architecture in T-ALL subtypes at diagnosis and relapse and will reveal new previously uncharacterized loci associated with T-ALL at different risk levels,

offering new markers of relapse and vulnerability targets for more effective therapeutic approaches.

Impact on cancer

Resistance to therapy is still a critical challenge in the context of acute leukemia. This study will provide an accurate picture of chromatin organization in leukemia development and relapse, allowing for better stratification of T-ALL patients and identifying / validating new possible therapeutic targets. Moreover, the knowledge database built during the study will foster the exploration of similar epigenetic regulatory mechanisms in other malignancies.

Luca Boldrini

Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Roma

MultiOmics MR guided radiothErapy Optimization in locally adVanced rectal cancER: the MOREOVER study

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Background

Complete response prediction in locally advanced rectal cancer patients is generally focused on the radiomics analysis of staging MRI. Omics information extracted from gut microbiota and ctDNA has never been integrated in composite biomarkers based models, leaving precious information out of the decisional process.

We here aim to integrate radiomics with gut microbiota and ctDNA based genomics tracking during neoadjuvant chemoradiotherapy (nCRT).

The MOREOVER study is a cutting edge proposal that stems out from an active trial and takes advantage of the unique technological park provided by the host institution, where a 0.35 T hybrid MR-Linac is active (only 3 units in Italy) and top level radiomics, microbiota, genomics and bioinformatics research facilities are available

Hypothesis

The main hypothesis that drives the MOREOVER study is that composite biomarkers will improve the current pCR predictive power of the radfiomics alone based modelling used in the THUNDER-2 trial (accuracy 90%; sensitivity 86%; specificity 92%; NPV 95%, and PPV 80%, AUC 0.93), paving the way towards a more accurate and comprehensive treatment personalization approach, thanks to the integration of actionable omics variables that may disclose previously unknown correlations with radiomics

Aims

Aims of this study are:

- to generate longitudinal microbiome data linked to disease resistance to nCRT and generate hypotheses about future therapeutic strategies in terms of both type of treatment and timing, such as fecal microbiota transplant in non-responding patients
- to describe the genomics pattern and ctDNA data evolution throughout the nCRT treatment in order to support the prediction outcome and identify new risk-category stratification agents
- To mine and combine collected data by integrated multi-omics approaches (radiomics, metagenomics, metabolomics, metatranscriptomics, human genomics, ctDNA) in order to increase the performance of the radiomics based response predictive model for LARC patients undergoing nCRT on MR-Linac

Experimental design

The MOREOVER project aims to enrich the phase II THUNDER-2 trial (NCT04815694) with gut microbiota and (ctDNA) omics information, by exploring the possibility to enhance the predictive performance.

Longitudinal ctDNA genomics, microbiome and genomics data will be analyzed on 7 timepoints: prior to nCRT, during nCRT on weekly base and prior to surgery. Specific modelling will be performed for data harvested, according to the TRIPOD statements.

Expected results

We expect to find differences in fecal microbiome, ctDNA and radiomics profiles between the two groups of patients (pCR and not pCR). In addition, we expect to find a variability in the stability of the considered omics features over time. The identified profiles will be inserted into dedicated modelling solutions to set up a multiomics decision support system able to personalize treatments.

Impact on cancer

The MOREOVER study is a great chance to improve the characterization of rectal cancer through extensive genomics

sequencing and microbiota analysis, paving the way to innovation and offering new paradigms of patients selection, toxicity reduction and risk stratification.

This will allow to efficaciously personalize cancer treatments, reducing the clinical, social and economic impact of overtreatments (i.e. surgery in completely responding patients), and allowing response enhancing strategies, such as microbiota transplant, radiation therapy boosting or genomic adjusted radiotherapy prescriptions.

Irene Caffa

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Endocrine therapy potentiation and mechanisms of drug enhancement in cancer via fasting

Background

Periodic fasting increases the activity of chemotherapeutics, tyrosine kinase inhibitors and immune-checkpoint inhibitors in mice. It also makes endocrine therapy for hormone receptor-positive (HR+) breast cancer (BC) more active and delays acquired endocrine resistance, while in parallel reducing adipose tissue and serum leptin (which are both remarkable effects given adipose tissue and adipokines' emerging role in BC pathogenesis and reponse to treatment). Fasting also holds promise for drug repositioning as it strongly enhances the antitumor effects to metformin, vitamin C and, as we recently found, of cholesterol biosynthesis inhibitors.

Hypothesis

We hypothesize that fasting delays acquired endocrine resistance in BC models by causing long-lasting epigenetic changes, by avoiding metabolic rewiring or changes in growth factor signaling in BC cells and/or by dampening the pro-oncogenic properties of adipose tissue; that combined fasting and cholesterol synthesis inhibitors act by blocking AKT signaling within plasma-membrane lipid rafts; and that high-throughput drug screens will reveal additional clinically-approved drugs that acquire anticancer properties via fasting, including agents that become exquisitely active towards cells expressing specific cancer mutations.

Aims

Aim 1: Defining whether fasting prevents endocrine resistance-inducing epigenetic changes, compensatory cell signalling pathways, metabolic reprogramming and adipose tissue features in BC cells.

Aim 2: Identifying the mechanisms for the cooperation between fasting and cholesterol biosynthesis inhibitors.

Aim 3: Identifying clinically approved drugs that acquire antitumor activity through fasting.

Experimental design

Epigenetic modifications, changes in cell metabolism, in growth factor signaling or in adipose tissue pro-tumorigenic features that promote endocrine resistance in BC xenografts in the absence, but not in the presence, of periodic fasting will be defined through RNA sequencing, ChIP-sequencing (for ER and active histone marks), by quantifying expression (through QPCR and Western blotting-WB) and post-translational modifications (e.g. phosphorylation; via WB) of specific proteins, by culturing BC cells with adipocyte-conditioned media, by protein silencing/overexpression in BC cell lines or by fat depot grafting and subsequent monitoring of BC xenograft response to endocrine agents. Cholesterol precursors in tumor xenografts and serum following treatment with fasting, cholesterol biosynthesis inhibitors or their combination will be measured by LC-MS/MS. In vivo add-back of circulating growth factors or LDL cholesterol will allow verifying their role in cholesterol inhibitor potentiation through fasting. We will transduce MCF7

cells with a PTEN-shRNA or with constitutively active, myristoylated AKT and HT29 cells with KRASV12: we will then probe these cells, together with the respective control cells, with collections of approved drugs w/ or w/o starvation conditions. Through follow-up experiments we will define the mechanisms for the newly-discovered fasting-induced drug sensitization phenotypes and evaluate them in vivo with tumor xenograft-bearing mice.

Expected results

We expect to i) identify epigenetic, metabolic or cell signaling-mediated mechanisms of endocrine resistance in HR+ BC, that are avoided by periodic fasting, ii) define the mechanism(s) underlying fasting-mediated enhancement of cholesterol inhibitor anti-tumour effects and iii) identify new clinically-approved agents that acquire anticancer effects through fasting.

Impact on cancer

This project will lay the basis for new approaches to treat cancer that take advantage of the broad metabolic effects of fasting and foster the repurposing of approved agents in oncology.

Carlo Cosimo Campa

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Mixing of Rab GTPase membrane domains reduces glioblastoma response to immunotherapeutic agents

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Background

Glioblastoma (GBM) is the most common and deadly form of brain tumour with no cure. The overall GBM incidence is 2-3 percent per 100,000 adults per year with a median survival of 15 months in patients who received aggressive radiotherapy and chemotherapy treatments. Attempts to improve GBM treatment using antibody-based therapeutic strategies did not provide a significant clinical benefit and numerous patients failed to respond.

Hypothesis

The potency of antibody-based therapies depends on their ability to induce the degradation of pro-tumorigenic cell surface proteins. Unfortunately, the endocytic recycling process prevents destruction of such cancer players and, consequently, cells escape from the therapy and they survive. As a matter of fact, elevated recycling rates in cancer cells reduce the efficacy of antibody-based therapies. Despite these evidences, we are still struggling to understand how pathological changes, such altered expression of endocytic recycling regulators, affect the efficacy of therapeutic treatments.

Aims

In this research proposal, we aim to define how genes involved in endocytic recycling reduce glioblastoma response to immunotherapeutic agents. To achieve this goal, we accumulated evidence showing that overexpression of Rab11 in GBM is involved in sensitivity of cancer cells to antibody-based treatments. We found that Rab11 function depends on Rab5, a regulator of endocytosis. In particular, we identified that this Rab5-Rab11 axis relies on the mixing of functionally distinct endosomal membrane domains decorated by either Rab5 or Rab11. Therefore, focusing on ABT-806 antibody and its drug-conjugate ABT-414, two distinct therapeutic treatment that failed in recent GBM clinical trials, we will address the role of the Rab5-Rab11 endocytic recycling axis in preserving the efficacy of such immunotherapeutic agents in GBM. The following tasks will be executed: (WP1) the role of Rab5-Rab11 membrane domains; (WP2) the role of Rab5-Rab11 recycling axis; (WP3) the role of Rab5, Rab11 and their interactors; (WP4) the predictive role of Rab5-Rab11 recycling axis in a GBM pre-clinical model.

Experimental design

To address this challenge, we will employ advanced technologies (i) to map the activity of Rab5-Rab11 recycling axis at subcellular resolution (FRET imaging) and, (ii) to reprogram the gene network controlling the Rab5-Rab11 axis (multiplexed orthogonal CRISPR/Cas genome engineering) in both patient-derived GBM cells and GBM preclinical model. This approach provides an invaluable opportunity to deeply investigate the role of endocytic recycling in GBM therapies.

Expected results

The proposed study will: (i) decipher the molecular and cellular basis of antibody-based immunotherapeutic

agents failure in GBM; (ii) identify novel biomarkers able to inform on the probability of patient response to antibody-based therapies; (iii) validate the predictive role of these biomarkers in a preclinical model of GBM; (iv) suggest ameliorations in both antibody and GBM clinical trial design.

Impact on cancer

GBM is an incurable disease with a median survival of 15 months and a 5-year survival rate of 5 percent. Antibody-based therapeutic strategies remain an unfulfilled promise for GBM treatment. The proposed study aims to identify novel predictive biomarkers to select patients that will benefit from these advanced immunotherapeutic agents.

Giulio Caravagna

Università degli Studi di Trieste, Trieste

Characterising genotype and phenotype clonal evolution of response to therapy with Artificial Intelligence.

Background

Therapeutic outcome of cancers depends on complex combinations of genotypes and phenotypes, which are shaped by evolutionary forces and therapy-induced selective pressures. The characterisation of disease relapse dynamics through sequencing of DNA and RNA of longitudinal samples can help understanding what drives treatment resistance, and tailor treatment to the patient's tumour at diagnosis. Unfortunately, the generation and integration of joint genotype and phenotype data poses challenges, and we still lack an evolutionary framework to integrate bulk and single-cell DNA/ RNA sequencing from longitudinal biopsies.

Hypothesis

In this project we posit to use Artificial Intelligence to integrate genotype and phenotype longitudinal measurements in the clonal evolution model by Nowell, and determine patterns of genetic and non-genetic evolution in response to therapy. We believe that AI can identify disease-specific evolutionary trajectories, and explain genotype-level with phenotype-level selective sweeps induced by treatment.

Aims

Creation of new AI that uses longitudinal biopsies to:
1) measure genetic patterns of clonal evolution from bulk DNA and RNA-sequencing;
2) measure plasticity patterns of clonal evolution from low-pass DNA and RNA sequencing of single-cells;
Our AI will be used to associate relapse-specific clonal evolution modalities for Acute Myeloid Leukaemia (AML) patients relapsing to allogeneic hematopoietic cell transplantation, and for Chronic Lymphocytic Leukaemia (CLL) patients relapsing to chemo-immunotherapy and the novel BCR inhibitor ibrutinib. We will assess the presence and predictability of relapse mechanisms from diagnostic samples, determining the extent of de novo post-treatment resistance.

Experimental design

We will collaborate with two AIRC-funded Units in IRCCS Hospitals (Hospital San Raffaele, Milan, and Centro Riferimento Oncologico, Aviano), and generate new bulk and single-cell data for AML and CD49d+ CLL patients. With bulk, we will work with bulk whole-genome DNA sequencing, and RNA sequencing of primary and relapse AML/ CLL biopsies. With single-cell, we will work with low-pass whole-genome DNA sequencing, as well as RNA sequencing of individual cells present at disease onset, and at relapse.

Expected results

Two orthogonal aims will deliver new integrative AI tools to study Cancer Evolution from longitudinal multi-omics cancer data collected during therapy. The AI will be used to characterise relapse modalities for two widespread haematological cancers, AMLs and CLLs, assessing resistance mechanisms due to genetic and plastic factors, identifying signatures of relapse predictable from diagnostic biopsies.

Impact on cancer

Many difficulties to treat cancer patients stem from our still superficial understanding of disease dynamics, and response to therapy. The clonal evolution paradigm by Nowell leverages Darwinian evolution to unravel mechanisms of tumour initiation and therapy response, relating clonal sweeps to selection and drugs-induced pressures. This project will integrate genotype and phenotype measures from longitudinal DNA and RNA sequencing using AI, producing technologies that can characterise different types of relapse dynamics, their evolutionary drivers and molecular profiles. With my clinical collaborators, we will unravel which evolutionary trajectories we should counteract to combat i) relapse in AML patients treated with allogeneic hematopoietic cell transplantation, and ii) relapse in CD49d+ CLL patients treated with chemo-immunotherapy and ibrutinib.

Luisa Carbognin

Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Roma

Prognostic and predictive role of cyclin-dependent kinase 4/6 pathway in breast cancer with lobular histotype.

Background

Invasive lobular cancer (ILC), represents the second most common type of breast cancer after invasive ductal carcinoma (IDC). The lack of prospective studies conducted in tumors with lobular histology (5-15% of all invasive breast tumors), has led oncologists to use the same prognostic factors commonly used in ductal tumors in clinical practice, and, consequently, to treat ILC patients as IDC, despite the overall differences in terms of clinical-pathological features and outcome. Thus, the molecular characterization of ILC, and its integration with clinical and pathological predictors of outcome, currently represents a research priority, in order to more precisely stratify ILC patients according to prognosis and to predict their potential susceptibility to specific targeted treatments.

Hypothesis

In order to explore the molecular characterization of 'pure' ILC, the current proposal will explore 2 research hypotheses: 1) the genomic abnormalities of the CDK4/6 pathway may be prognostic in ILC patients receiving endocrine therapy (ET); 2) the dysregulation of the CDK4/6 pathway may predict resistance to CDK4/6 inhibitors (CDK4/6i) in metastatic LC patients treated with CDK4/6i plus ET. These hypotheses are supported by: 1) our preliminary results, suggesting that copy number gain of CDK4 (or alterations in CDK4/6 pathway) may negatively influence LC prognosis; 2) early findings indicating that genomic alterations in CDK4/6 pathway seem to be associated with resistance to CDK4/6i and ET.

Aims

The specific aims of the current project will allow:

1) To evaluate the impact of molecular factors (with particular regard to CDK4/6 pathway) in determining the prognosis of ILC patients;

2) To characterize and validate the previously identified prognostic molecular drivers in the pre-clinical setting (ILC cell lines and mouse models), exploring also the potential therapeutic efficacy of a targeted approach with CDK4/6i;

3) To evaluate the predictive impact of molecular alterations (involved in the cell-cycle regulation) in metastatic LC patients treated with endocrine therapy plus CDK4/6i.

Experimental design

The project will be performed through 3 phases (Work-Packages):

1) Retrospective collection of ILC samples with clinical-pathological annotations in different disease setting, in order to perform genomic/transcriptomic analysis with next generation sequencing;

2) Establishment of ILC cell culture and orthotopic mouse model of human ILC for in vitro and in vivo assessment of the identified prognostic molecular drivers and the potential therapeutic efficacy of a pharmacological approach;

3) Conduction of a prospective, proof of concept biomarker-stratified study in metastatic ILC patients, candidates to receive a first-line treatment with ET plus CDK4/6i. Patients' tissue and blood samples will be collected and analyzed by integrated genomic and proteomic analyses.

Expected results

The expected results will be:

1) To validate the potential prognostic role of CDK4/6 pathway alterations in ILC; 2) To identify predictive factors of resistance/response to CDK4/6i plus ET in metastatic LC patients.

Impact on cancer

This project may contribute to fill a need for clinical practice represented by the identification of predictors of prognosis and treatment efficacy for ILC to assist in the choice of a pertinent therapeutic decision.

Carmine Carbone

Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Roma

Molecular profiling to detect early-steps of pancreatic carcinogenesis to improve prognosis and clinical outcome of IPMN

Background

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease and is projected to be the second cause of cancer death in Western societies within a decade. It arises from non-invasive precancerous lesions, including pancreatic intraepithelial neoplasia (PanIN) and intraductal papillary mucinous neoplasm (IPMN), which are curable if detected and treated before they progress to invasive carcinoma.

Progression of PanIN into invasive cancer has been well characterized, while there is an urgent need to understand the biology of IPMNs. IPMNs present a unique clinical challenge, as they are very frequent and incidentally found in about 13% of subjects who undergo routine abdominal imaging studies.

Some IPMNs have a very high cancer risk (malignant (M-IPMN) and do require surgery, while others are low-risk lesions (non-malignant (NM-IPMN) that can be followed-up clinically. Molecular mechanisms beyond IPMN degeneration are still unknown. Establishment of IPMN organotypic cultures represents the best model to interrogate molecular pathways to possibly elucidate the underlying mechanisms of IPMN degeneration and pancreatic carcinogenesis.

We firmly believe that cell-derived in vitro 3D-organotypic cultures will allow to study biological processes, such as cell behaviour in an environment that mimics endogenous cell organization and organ structures.

Hypothesis

We hypothesize that molecular comparison of NM-IPMN, M-IPMN and tumour-associated IPMN may lead to the identification of mechanisms responsible for pancreatic carcinogenesis. This will lead to considerable progress in IPMN decision making process, providing new tools for early detection of potential degenerating IPMN, as well as, molecular based rational for addressing patients to the most appropriate therapeutic management.

Aims

The AIM of the present proposal is to identify and validate biomarkers responsible for degeneration of IPMN and the early metastatic dissemination of IPMN-derived PDAC in order to stratify IPMN patients to different therapeutic interventions.

Experimental design

We plan:

A) To establish models of IPMN pathogenesis and degeneration through the creation of two training set of patients. 1) A prospective cohort of IPMN patients for blood and tissue collection and establishment IPMN organotypic cultures; 2) A retrospective cohort of surgically resected IPMN patients with available fixed tissue;

B) To identify the pathways and molecular profile differentially regulated in NM-, M- and tumor-associated-IPMNs;

C) To confirm in in vitro and in vivo models the molecular mechanisms of IPMN carcinogenesis previously identified;

D) To validate the mechanisms of IPMN degeneration in the retrospective validation set of IPMN patients who underwent surgical resection.

Expected results

With our proposal, we expect to identify fundamental biomarkers to discriminate between M- and NM-IPMNs in order to provide new tools able to drive management decision and to propose novel potential therapeutic targets for these patients.

Impact on cancer

This proposal will provide novel insights in the understanding of the mechanisms beyond progression of IPMN to invasive PDAC. Most importantly, we will able to disclose new molecular biomarkers of IPMN degeneration that can be potentially developed to further stratify these patients. The results of this study will reflect in a change of clinical practice, making possible to predict patients who will progress from those who will not even need to be followed up.

Debora Carpanese

IOV - Istituto Oncologico Veneto - I.R.C.C.S., Padova

Hyaluronan as an effective immunological adjuvant for the creation of protein-based vaccines against HER2 breast cancers

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Background

Protein-based vaccines represent a balance between a good safety profile and a relatively rapid manufacturing, but require efficient adjuvants to overcome their weak immunogenicity. We recently demonstrated and patented that the conjugation of protein antigens (Ags) to fragments of hyaluronan (HA) likely acting as toll-like receptor (TLR) 2/4 agonists, leads to robust and long-lasting Ag-specific immune responses in different mouse strains and in both infant and aged mice, suggesting that this technology might be particularly advantageous for poorly responding subjects such as cancer patients.

Hypothesis

Overexpression of the human epidermal growth factor receptor 2 (HER2) occurs in 15-30% of breast cancer (BC) cases and is considered an adverse prognostic factor, with 20% of patients with HER2+ early BC experiencing relapse and developing advanced disease. Anti-HER2 vaccines could be employed in a therapeutic setting alone or in combination with other therapies, to target early stages of disease or to prevent the development of metastases.

Aims

Willing to exploit the potentiality of our HA-based vaccination approach in the context of a real disease model, we aim at developing and studying the efficacy and safety of HA-conjugated protein and peptide-based candidate vaccines against HER2-expressing BCs, as well as elucidating the mechanism of action underlying HA adjuvanticity. Peptides will be selected among the most immunogenic sequences that are retained in rat, mouse and human HER2 (universal peptides), able to bolster HER2-specific CD4+ and CD8+ responses.

Experimental design

The efficacy of vaccine prototypes will be assessed and compared with clinically relevant adjuvants/peptides comparators. The magnitude and longevity of the humoral and cellular Ag-specific immune responses will be assessed in both the preventive, preneoplastic and therapeutic settings, using different mouse strains and transgenic models. The best performing prototypes will be also investigated in combination therapies with clinically-relevant options. To pinpoint the mechanism of action responsible for HA adjuvanticity, different mouse strains and transgenic mouse models will be employed, together with in vivo depletion of immune cell subsets. Quantitative biodistribution of HA-vaccines and the involvement of LYVE-1 receptor will be assessed by radioimaging, while the major immune cell players involved will be deciphered both in vitro and in vivo using multiple techniques such as quantitative multispectral digital pathology microscopy, gene expression analysis, confocal microscopy, and flow cytometry.

Expected results

We expect to characterize and validate at least one HA-based vaccine prototype that is capable of strongly

interfering with the growth of HER2+ BC. The concomitant thorough assessment of the mechanism of action and toxicology will support the rapid transfer from the bench to bedside, and the scale up to clinical trials.

Impact on cancer

Cancer is a major public health problem worldwide, and the recent COVID-19 pandemic demonstrated how its management can be hampered in situations of global public health emergencies. The peerless versatility of our HA-based vaccine technology might have an extremely significant social impact at different levels, as it would offer new solutions for both preventing the development of different hereditary neoplasms and providing new effective weapons for the therapy of existing neoplasms and relapses of disease.

Matteo Cereda

Università degli Studi di Milano, Milano

Deciphering alternative splicing deregulation in cancer to identify novel therapeutic targets

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Background

Alternative splicing (AS) is the fundamental process that drives proteome diversity in the majority of human genes. This mechanism is finely regulated by RNA-binding proteins (RBPs) and alterations of this regulation can lead to diseases, including cancer. During the last decade, cancer genome projects have released thousands of sequencing data to describe the genetic alterations of tumours. Despite the availability of these data, a characterisation of AS deregulation across and within cancer types, and its effect on cancer onset and progression, is still missing. Therapeutic intervention targeting AS has been extensively reviewed as a promising way to treat cancer but its development is still limited.

Hypothesis

Our working hypothesis, sustained by our preliminary results, is that the expression of RBPs is altered in a large proportion of cancer types and patients. This somatic aberration leads to a defective splicing that promotes and sustains cancer.

Aims

Our goal is to systematically assess AS deregulation in cancer to uncover novel potential therapeutic targets. We intend to evaluate the heterogeneity of cancer-promoting AS events across and within different cancer types. Our specific aims are: (1) to determine the role of RBPs in cancer progression, (2) to identify novel actionable targets, (3) to assess the causative mechanisms of defective AS regulation, (4) and provide a list of tested splicing switching oligonucleotides (SSOs) able to target oncogenic AS events and block the progression of the tumour.

Experimental design

We will analyse sequencing data available at international consortia of 14,551 tumours of 39 cancer types to identify RBPs responsible for defective splicing, their associated oncogenic AS events and their causative somatic alterations. We will corroborate the bioinformatics results with genomic and biochemical approaches in controlled cell systems, where we will recapitulate the defective AS and measure the phenotypic response. We will design SSOs for candidate oncogenic AS event and evaluate their ability to block tumour progression using in vitro and in vivo experiments on cell lines.

Expected results

We expect to: (1) generate an integrated and harmonized dataset of somatic alterations within/across cancer types; (2) identify and characterize cancer-type-/patient-specific deregulated RBPs, AS events, and causative mechanisms; (3) determine and validate novel cancer driver alterations; (4) assess the intra-/inter-tumour heterogeneity of AS regulation; (3) provide a repertoire of experimentally validated SSOs able to block the progression of the tumour.

Impact on cancer

Assessing AS deregulation in cancer will allow to identify novel oncogenic alterations, where somatic variations (e.g. mutations, copy number variations) might be absent. As a result, protein involved in AS as well as regulators and modulators of AS might become new drug targets, and thus could be instrumental for the design/delivery of novel personalized therapeutic treatments.

Chiara Ciccarese

Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Roma

Characterization of patterns underlying the crosstalk of resistance to anti-VEGFR and immunotherapy in kidney cancer

Background

The standard first-line therapy for metastatic renal cell carcinoma (mRCC) includes either combinations of an immune checkpoint inhibitor (ICI) plus an anti-angiogenic agent (VEGFR-TKi), or dual ICIs targeting PD-1 and CTLA-4 (i.e. nivo+ipi). Despite ICI-based combos significantly improve PFS and OS, the majority of patients progress within 18 months. Two opposite approaches have been investigated to delay tumor resistance: a) intensification adding a VEGFR-TKi to nivo+ipi (triplet therapy), with improved antitumor activity however at the cost of increased toxicity; b) VEGFR-TKi discontinuation, with preliminary experiences leading to similar life expectancy and better safety profile. Our research group has actively contributed to investigate these personalized strategies; TIDE-A study evaluates a "de-intensification" approach (VEGFR-TKi discontinuation in case of tumor response to VEGFR-TKi+ICI) while AxIn trial will assess an "intensification" regimen (a VEGFR-TKi added to nivolumab after nivo+ipi). Molecular exploratory analyses support the idea of different tumor phenotypes associated with a prevalent angiogenic or immune signature, but no predictive biomarkers have been identified so far.

Hypothesis

We hypothesize that biomarker analyses of tumor and plasma samples of three mRCC groups of patients based on response to therapy (angiogenesis-dependent, immune-responder, non-responder) within TIDE-A and AxIn studies, and comparisons with the validation real-world cohort (APAChE-2 study), could lead to the identification of morphological, molecular, and biohumoral signatures underlying the dependence on angiogenesis or immune-responsiveness in order to predict benefit/resistance to immunotherapy or anti-angiogenic therapies.

Aims

We aim to identify and validate morphological features, specific gene alterations, and biohumoral signatures that predict response/resistance to VEGFR-TKi, ICI or both in mRCC so as to better tailor the first-line therapy

Experimental design

We plan to identify molecular pathways differentially associated with response or resistance to immunotherapy and anti-angiogenic therapy by:

1) Characterizing tumor morphology with IHC and digital pathology with artificial intelligence

2) Identifying specific gene alterations (mutation, SNPs and CNVs) with NGS

3) Studying spatial landscape of tumor- immune and vessels cells interaction in order to identify specific cell population (neoplastic and microenviroment cells) and putative determinants of resistance/sensitivity

4) Identifying changes in circulating immune-related cytokines of immune activation/evasion, and signatures related to angiogenesis, hypoxia, cell proliferation, and inflammation by single cell spatial transcriptomics.

We will compare the three groups of patients (non-responders vs. angiogenesis-dependent vs. immune-

dependent) within the three different studies populations (TIDE-A and AxIn as discovery cohorts, APAChE-2 as validation cohort).

Expected results

The current project is expected to provide a breakthrough in the management of mRCC through a precision medicine-based approach that relies in the identification of patients more likely to benefit from ICI-based combos that also include anti-angiogenic agents rather than dual ICIs.

Impact on cancer

The availability of prospective cohorts of patients treated with innovative intensification/de-intensification strategies represents an unprecedented opportunity for identifying molecular mechanisms of dependence/resistance to VEGFR-TKi or ICI, leading to a personalized molecular-based therapeutic approach. This project might have relevant impact, helping to better select patients for the most adequate therapy, improving the chance of cure, delaying the start of other anticancer therapies, and avoiding unnecessary toxicities.

Francesco Cucco

Consiglio Nazionale delle Ricerche, Pisa

FUNCTIONAL CHARACTERIZATION AND DIAGNOSTIC/PROGNOSTIC IMPACT OF MYC POINT MUTATIONS IN DLBCL

Background

Diffuse large B-cell lymphoma (DLBCL) is the most common haematological malignancy and accounts for more than 80% of high-grade lymphomas. MYC translocation (MYC-R) occurs in 10% to 15% of DLBCL. In DLBCL, MYC-R is associated with different partners and those with an immunoglobulin (IG) gene confer inferior clinical outcome. The reason for this is unclear. We identified highly frequent MYC point mutations in DLBCL cases with a MYC/IG genes translocation (~65%). The nature of these mutations is uncertain. Most likely they are caused by the aberrant somatic hypermutationmachinery (aSHM) activity, but their functional impact on MYC and cells biology is still largely unknown.

Hypothesis

MYC is frequently deregulated in cancer, mostly due to amplification and translocation events. MYC point mutations have been found in a variety of solid and non-solid cancers, but only few of them have been functionally tested so far. These mutations could have a previously underestimated impact on cancer evolution, particularly in DLBCL, where they could act in synergy with MYC/IG translocation. Therefore, these mutations can be potentially used as prognostic/diagnostic biomarkers and drive treatment decision in the clinic.

Aims

•To identify and expand the spectrum of pathogenic mutations that enhance MYC protein stability and trigger cell transformation.

•To investigate all relevant MYC mutations for further characterization in vitro and in vivo.

•To comprehensively assess the prognostic value of MYC translocation/mutation in a large cohort of DLBCL MYC-R cases.

•To evaluate if MYC mutations can be used for the early detection of the high-risk DLBCL with MYC/IG gene translocation at the time of diagnosis

Experimental design

In the first part of the present proposal, we will perform in vitro and in vivo experiments employing different cell lines and animal models in order to functionally characterize the MYC point mutations previously identified in DLBCL MYC-R cases. The second part of the investigation will be focused on the molecular characterization of DLBCL samples. This will be achieved by using fluorescent in situ hybridization (FISH) and a combination of sequencing approaches like Digital Droplet PCR (ddPCR) and Next-Generation Sequencing (NGS).

Expected results

By the end of the project, we anticipate to have a full picture of the impact of the investigated MYC point mutations in terms of enhancing MYC protein stability, transformation capacity and tumorigenesis.

Moreover, the correlation of the MYC mutation status with different molecular and clinical data in a large cohort of DLBCL MYC-R cases will build up an integrated pathway for the prognostic stratification of these cases that might be used in a routine clinical setting.

Impact on cancer

This work will shed light on the role of MYC point mutations in B cell malignancies. It will also elucidate the translational potential of the relevant MYC point mutations, defining new prognostic markers for high risk DLBCL patients, improving diagnosis, and establishing new targeted therapies. On a larger scale, this work also has the potential to be extended to other malignancies sharing similar genetic features.

Sara De Biasi

Università degli Studi di Modena e Reggio Emilia, Modena

Unravelling the role of mucosal associated invariant T cells in non-small cell lung cancer

Background

Circulating mucosal associated invariant T (MAIT) cells are an innate-like pro-inflammatory and cytotoxic population of effector memory T cells and can represent up to 10% of peripheral CD8+ T cells. They recognize microbial proteins presented by non-polymorphic MHC class I related-molecule (MR1), display homing properties and are characterized by the expression of semi-invariant Va7.2 T cell receptor (TCR) combined with high level of the inhibitory receptor CD161. MAIT cells are deeply involved in orchestrating the immune response in the mucosae. Controversial data exist regarding the role of MAIT cells in cancer. We recently showed that circulating MAIT cells predict response to anti-PD1 therapy in metastatic melanoma patients and they reside in the tumor microenvironment (TME), at higher percentage in responding patients. MAIT cells are enriched also in tumor lesions of non-small cell lung cancer (NSCLC) patients, but scanty data exist on cell-cell interactions and mechanism(s) of function of these cells in the TME. Recently, a rare population of MAIT cells has been depicted as capable to respond to tumor cells of different tissue origin.

Hypothesis

Based on recent preliminary and unpublished data, MAIT cells play a cancer-specific cytotoxic role in NSCLC and show potential predictive value for recurrence. The main aim of the study is to better depict the identity of MAIT cells, to identify their function and role within the TME of resectable NSCLC, and to unravel the mechanisms and interactions at the basis of their function.

Aims

We plan to profile and modulate this population in NSCLC, and in particular to: 1. provide new information on the structure, organization, and relationship of the immune and tumor synapses; 2. understand if they are in fact main actors of the immune response; 3. identify the rationale and develop possible tools for modulating their action.

Experimental design

In this five-year project, on a total of 80 patients with resectable NSCLC, we will: 1) broadly characterize MAIT cells in the TME and their immune synapses, by investigating their spatial localization and interaction, decipher their phenotypic, functional and metabolic profiles and correlate immunological data with clinical outcome; 2) evaluate MAIT cells response to tumor associated antigen (TAA); 3) assess a rationale for MAIT function modulation by interfering with the CD161-LTT1 axis in vitro, decipher an anti-cancer immune response of MAIT cells in lung cancer tumor-spheroid-MAIT-cell coculture system and reprogram and redifferentiate MAIT cells (reMAIT).

Expected results

We will expect to clarify the role of MAIT cells, finding their main interactors in TME and quantify their anti-cancer response. We expect to pinpoint new regulating mechanisms of cytotoxicity exerted by MAIT cells

and to identify new MAIT-related features associated with clinical outcome of NSCLC.

Impact on cancer

The 5-year survival of NSCLC patients is low. For this reason, given that MAIT cells are cytotoxic, reluctant to cause graft-versus-host disease, are cancer-drug resistant (they express multidrug efflux pump such as CD243) and reinforce expansion and the effector functions of NK cells in tumor, the advent of induced pluripotent stem-cell-derived MAIT cells (reMAIT cells) will make it possible to harness these cells for immune cell therapy.

Marco De Giovanni

Università Vita-Salute San Raffaele, Milano

The role of GPR35 in the context of myeloid cell migration to tumors and immunotherapy

Background

Efficient immune responses to tumors rely on the synchronized migration of leukocytes between and within tissues. Immune cell migration depends on leukocyte sensing of directional cues by surface receptors named G-protein coupled receptors (GPCRs). GPCRs are druggable and control many aspects of cancer immunity and tumorigenesis, including immune cell migration, tumor proliferation and invasion. Despite their ability to regulate a broad range of key functions in tumors, there are few approved compounds targeting these receptors in the context of cancer, and the role of GPCRs in tumor progression remains elusive. Thus, understanding how GPCRs regulate anti-tumor immunity is essential to design innovative therapies. Here we propose to analyze the role of GPR35, a pro-migratory GPCR, in the context of immune cell recruitment to tumors. We have recently showed that GPR35 drives neutrophil endothelial adhesion and transmigration, in response to serotonin metabolite 5-hydroxy-indole-acetic acid. GPR35 is abundantly expressed in tumor-associated immune cells, and it was suggested to promote cancer progression. Our preliminary observations indicate that GPR35 sustains tumor progression and shapes tumor-associated myeloid cell infiltration.

Hypothesis

Recruitment of pro-tumorigenic immune cells depends on endothelial adhesion and transmigration. Accordingly, we speculate that GPR35 sustains recruitment of pro-tumorigenic myeloid cells to support cancer progression.

Aims

Our aim is to answer two main questions: 1) does GPR35 shape myeloid cell recruitment, migration, positioning and interaction within tumor microenvironment, and what is the impact of these events on disease progression? 2) What is the role of GPR35-expressing immune cells in serotonin modulation of immunotherapy, and can GPR35 influence tumor response to immune checkpoint blockade?

Experimental design

We will analyze the role of GPR35 in immune cell recruitment to tumor by advanced imaging techniques (histocytometry, 2-photon intravital microscopy, CODEX, NICHE-seq) and flow cytometry in cancer models in which GPR35 expression was reported to be elevated (melanoma, colorectal carcinoma and hepatocellular carcinoma). To study the behavior of GPR35-sufficient and deficient cells within the same tumor, we will analyze cell accumulation and positioning within tumor-bearing irradiated mixed chimeras, where 50% of bone marrow derived cells will either be sufficient or deficient for GPR35. In addition, we will track tumor growth and survival in GPR35WT and KO mice over time, to assess the impact of GPR35 on disease progression and immune checkpoint blockade efficacy.

Expected results

We believe that our experimental approach will unravel the role of GPR35 in the context of immune responses to tumors. Indeed, we speculate that GPR35 sustains recruitment and influences intra tumor positioning of pro-tumorigenic myeloid cells to sustain cancer progression. In addition, we anticipate that the absence of GPR35 may indirectly influence anti-tumor T cell responses to immune checkpoint blockade.

Impact on cancer

Taken together, these observations indicate that GPR35 may represent an important pro-tumorigenic factor and attractive target to therapeutically alter immune cell infiltration in the context of cancer and immunotherapy. Given this receptor-ligand pathway can be targeted by available compounds, we believe our research to have a potential high impact on cancer therapy within 5 years.

Loretta Laureana del Mercato

Consiglio Nazionale delle Ricerche, Lecce

Nano-patterned metastatic melanoma for quantifying metabolic changes in mediated drug resistance

Background

In recent years, there has been considerable progress in in vitro cell cultures to create tumors in a dish. Nowadays, we can take a tumor sample from the patient and dissociate it into its functional units that are subsequently transferred in a gel matrix to initiate organoid culture under appropriate conditions [Drost, Nat Rev Cancer 2018]. Several studies assessed that the genetic expression, the protein expression, the cell types and morphologies found in the host tumors are replicated in organoids [Boj, Cell 2015]. However, the majority of studies employ static analyses that only provide bulk measurements of the processes occurring within the 3D systems. For instance, cellular metabolism, that is recognized a surrogate measure of drug response, is measured by standard assays that measure mitochondrial respiration and glycolysis by quantifying the O2 consumption and extracellular acidification rates of 3D cultures in multi-well plates.

Hypothesis

Averaging everything is happening through the whole 3D model do not permit to distinguish between different subtypes of cells in a heterogeneous cell population. The spatial mapping of physiological parameters influencing the cell metabolism within in vitro tumoroids is at present time highly challenging.

Aims

Generating an efficient method for testing the metabolic activity of tumoroids using 3D sensing scaffolds that can measure the metabolic changes of tumoroids with high spatial/temporal resolution, going beyond the possibilities offered by current standard assays.

Experimental design

1) Realization of BRAF wt and BRAF or RAS mutated tumoroids of metastatic melanoma cell lines including tumor microenvironment components, such as immune cells (lymphocytes, macrophages, and dendritic cells) and fibroblasts, and optical sensors for spatio-temporal read-out of lactate and O2.
2) Studying tumor microenvironment impact on drug response by sensing lactate and O2 in the whole 3D model during therapy. Cell metabolic phenotypes will be extracted from the obtained maps and their changes will be correlated to the tumor microenviroments dynamics.
3) Standardization of sensing tumoroids initiated by primary tumor cells from tissues samples of MM patients. Real-time mapping of tumour growth measuring lactate and O2 gradients across the 3D model. Evaluation of drug(s) response and analysis of cellular metabolic changes in response to conventional chemotherapy and/or targeted drugs (e.g., BRAF inhibitors, MEK inhibitors). The follow-up of donor patients will allow the comparison of the in vitro results with their clinical response to therapy in order to validate the predictive power of the MM tumoroids systems.

Expected results

1) To non-invasively map lactate and O2 gradients in cell aggregates of in vitro MM models.

2) To study how cell-cell and cell-environment interactions influence the levels of lactate and O2 during therapy.
3) To assign physiological significance to the measured changes by implementing proper computational tools.
4) To identify therapies that affect all cells in a heterogeneous melanoma.

Impact on cancer

The direct and non-invasive spatial/temporal mapping of physiological parameters influencing the cell metabolism and their response to drugs in a 3D environment, with sub-micron resolution, will provide substantial advancements in assessing the efficacy of anticancer therapies as well as in measuring drug resistance in patient-derived MM models.

Eros Di Giorgio

Università degli Studi di Udine, Udine

A druggable approach to modulate cancer epigenetics

Background

The incidence of Colorectal cancer (CRC) in Friuli-Venezia Giulia, the region where the proponent will carry-on this project, is the highest in Italy. Despite the progress achieved in the treatment of primary CRC, only partial results have been obtained in the treatment of metastases.

Hypothesis

The genetic model that successfully describes the development of CRC neoplasm failed to predict the formation of tumor subclones that evolve into treatment-resistant metastases, as a consequence of random genetic events and the exposure to massive genotoxicity. In the context of third generation therapies, the reprogramming of anti-tumor immunity and of cancer epigenetics are achieving promising results.

Aims

Here the PI aims to create an epigenetic atlas of primary and metastatic CRC and to identify the epigenetic changes that feature metastatization and refractoriness to conventional therapies. Moreover, the applicant aims to reprogram CRC epigenetics to selectively increase the genetic instability and immunogenicity of metastases and make them sensitive to treatments and anti-tumor immunity.

Experimental design

For this purpose, antagonist complexes (HDAC4-HDAC3 and p300-BRD4) that control chromatin acetylation will be selectively targeted by using small molecules and loss-of-function approaches. The strength of this epigenetic reprogramming in increasing the genetic instability and the sensitivity of CRC metastases to conventional therapies will be tested on 2D CRC cellular models and 3D patients' derived organoids (PDOs). The perturbations on CRC transcriptome and epigenome will be recorder by high-throughput techniques (RNA-seq, ChIP-seq, Repli-seq) assisted by advanced systems of protein complexes purification (CLASP, MudPIT). Moreover, the effectiveness of a recently developed HDAC4 inhibitor in stimulating anti-cancer immunity will be tested on syngeneic models and compared to already available epigenetic drugs.

Expected results

The results collected through this study will allow to understand how much the targeted alteration of CRC Epigenetics influences its genomic stability/instability and it is successful in re-educating cancer cells to respond to conventional and new generation therapies. Finally, an atlas of the epigenetic changes observed during CRC progression, remission and response/refractoriness to therapies will be compiled and immediately tested for its ability to predict disease evolution and patients' therapeutic response.

Impact on cancer

In addition to achieving these ambitious goals, the PI aims to lay the foundations for developing a personalized epigenetic cancer therapy approach sustainable also on a regional basis.

Rosa Di Micco

Università Vita-Salute San Raffaele, Milano

Tailoring treatment of luminal A and lobular breast cancer with 18F FES-PET/MRI

Background

Breast cancer (BC) management has evolved towards a tailored treatment mainly based on tumor biology and patient's characteristics. Staging at diagnosis is crucial to build up the most appropriate therapy. However, imaging performance is not equivalent in all subtypes. In particular, staging of luminal A BC(LumA) and lobular BC(Lob) remains complex. On one side, LumA defined as being estrogen receptor(ER)- and progesterone receptor-positive, well-differentiated, Her2-negative with a low proliferation index (< 20%) has worse sensitivity on axillary US, reduced MRI enhancement and low FDG-avidity on PET. On the other side, Lob presents a peculiar growth pattern that makes MRI less sensitive on axillary and systemic staging and FDG-avidity lower. Despite the relatively favorable prognosis, nodal metastases and recurrence are possible and, as LumA and Lob account for >50% of all BCs, their absolute values are higher compared with other subtypes with a concrete risk of disease underestimation and undertreatment. Genomic testing showed that a high genomic risk of recurrence could be hidden even behind low clinical risk tumors. To date, obtaining an initial reliable staging of LumA and Lob in order to plan the most appropriate treatment pathway and to minimize the recurrence risk still represents a partially unmet clinical need. In San Raffaele Hospital, two ongoing studies on FDG-PET/MRI in BC staging showed that its sensitivity decreases in these BC types.

Hypothesis

Our hypothesis is that combining the advantages of hybrid PET/MRI and the high sensitivity/specificity of 16-alpha-18F-fluoro-17-beta-estradiol(FES), a radiolabeled form of estrogen binding to functionally active ER, we could obtain a reliable, non-invasive, operator-independent, one-stage imaging method for staging LumA and ER-positive Lob.

Aims

Aim 1: Evaluating the performance of FES PET/MRI in axillary staging compared with axillary surgery.
Aim 2: Evaluating potential correlations between changes in FES uptake and changes in proliferation index after three weeks of endocrine therapy before surgery.
Aim 3: Evaluating the performance of FES PET/MRI in systemic staging of patients undergoing systemic therapy in comparison with standard imaging.
Additionally, biological determinants of tumor heterogeneity will be investigated.

Experimental design

This is a prospective cohort study where patients with LumA and ER-positive Lob will be enrolled in four cohorts undergoing: primary surgery; induction endocrine therapy; neoadjuvant chemotherapy; systemic therapy for metastatic disease. For the purpose of the study an additional FES PET/MRI exam will be performed at baseline for local and systemic staging and a second exam after systemic therapy. Correlations between FES PET/MRI parameters and pathology, gene expression, CTCs, and FDG PET parameters, when available, will be investigated.

Expected results

We expect that results from this prospective trial will allow a personalized BC staging in LumA and Lob providing evidence of FES PET/MRI performance in different settings: early and advanced BC, response prediction and monitoring.

Impact on cancer

Studying the performance of FES PET/MRI in selected BC cohorts will have an important impact on the stratification of BC patients, allowing a customization of surgery, radiotherapy and systemic therapy, and to potentially improve patient prognosis. Furthermore, using a non-invasive molecular and functional imaging could positively affect healthcare system reducing direct and indirect BC-related costs.

Diego Dibitetto

Istituto di Ricerche Farmacologiche "Mario Negri" I.R.C.C.S., Milano

A Proteo-Genomic Approach to Study DNA-PK's Function in PARP Inhibitor Resistance

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Background

DNA-PKcs is a kinase with key roles in DNA double-strand break repair. Recently, additional roles for DNA-PKcs have been described in RNA metabolism and activation of interferon response, but the underlying mechanisms have not been elucidated. DNA-PKcs is frequently over-expressed in advanced cancers and, for this reason, DNA-PKcs inhibitors have been developed and are currently being tested as adjuvant agents in different oncologic patients. However, it is currently unclear how DNA-PKcs inhibitors exacerbate the cellular sensitivity to chemotherapy and which oncologic patients may benefit from these drugs as adjuvant therapy.

Hypothesis

In this research proposal, we will focus on a non-canonical function for DNA-PKcs kinase in preventing genomic Single Strand Break (SSB) accumulation. Consequently, I have found that impaired DNA-PKcs function generates a novel cellular vulnerability, which is the sensitivity to PARP inhibitors. The DNA-PKi-induced vulnerability to PARPi is further exacerbated in cells lacking BRCA1/2 function and can be therapeutically exploited to overcome acquired PARPi resistance in breast and ovarian cancer patients.

Aims

Throughout this research proposal, we will map the genome-wide localization of SSB in cells lacking DNA-PKcs function and functionally characterize these genomic subdomains. Moreover, we will narrow down to individual DNA-PKcs phosphorylation targets involved in SSB suppression and PARPi resistance. Finally, we will establish high-grade serous ovarian carcinoma-derived organoids from patients with somatic and germline BRCA1/2 mutations characterized by resistance to the clinically-approved PARPi Olaparib. Using these models, we will translate the accumulated knowledge and rationally use DNA-PKcs inhibitors to restore PARPi sensitivity.

Experimental design

To achieve my research aims, we will initially combine the ChIP-Seq and S1 END-Seq techniques to profile the genomic subdomains occupied by DNA-PKcs and map the genomic sites where DNA-PKcs suppresses SSB accumulation. To identify the precise DNA-PKcs effectors involved in SSB suppression and PARPi resistance, we will use phosphoproteomics to discover new DNA-PKcs phosphorylation targets in PARPi-resistant mammary tumor cells and then we will functionally and genetically characterize how DNA-PKcs-mediated phosphorylation regulates protein's function. Finally, we will establish de novo 3D patient-derived organoid models from BRCA-mutated high-grade serous ovarian carcinoma patients with acquired PARPi resistance and use DNA-PKcs inhibitors to overcome PARPi resistance.

Expected results

Results from this research action are expected to reveal unprecedented roles for DNA-PKcs in genomic stability different from its canonical role in DNA repair. With our designed proteo-genomic approach, I expect

to characterize a new signaling mode for DNA-PKcs and identify previously unknown phosphorylation targets important for SSB suppression and PARPi resistance. Finally, based on our preliminary results obtained with PARPi-resistant mammary tumor cells, I expect likewise we will be able to overcome PARPi resistance in patient-derived ovarian cancer organoids lacking BRCA1/2 function with the use of DNA-PKcs inhibitors.

Impact on cancer

PARPi resistance is an emerging clinical challenge, particularly for germline mutated BRCA1/2 patients with recurrent breast and ovarian cancer. In this proposal, we have used DNA-PKcs inhibitors as a novel approach to overcome PARPi resistance in BRCA1/2-deficient tumors. I believe this new concept represents a breakthrough contributing to improve the quality of life of several oncologic patients in the coming years.

Gabriele Matteo D'Uva

Istituto Nazionale di Biostrutture e Biosistemi, Bologna

Dissecting the cross- regulation between EGFR and ERBB2 in basal-like breast cancer

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Background

These past decades have witnessed the impressive advances in the comprehension of the role of growth factor receptors of the ErbB family in the development and progression of various solid cancers. Indeed, targeting ErbB2 is a milestone in HER2+ metastatic breast cancer treatment. EGFR has been associated with basal-like and triple-negative breast cancers; however, clinical trials evaluating the efficacy of anti-EGFR drugs in these subtypes have led to disappointing results.

Hypothesis

We observed a robust association between higher EGF/EGFR levels and better relapse-free survival in basal-like and triple-negative breast cancer patients. Although these data may appear in contrast with a large body of data supporting an oncogenic role for EGFR, a paradoxical reduction of EGFR abundance in breast cancer metastasis has been already reported in mouse models. In the context of the basal-like breast cancer, we here hypothesize that exposure to high EGF doses reduces ERBB2 expression, and through the activation of EGFR/EGFR homodimers promotes cell differentiation, thus reducing metastasis. On the other hand, low EGFR signaling would facilitate ERBB2 expression, the transition towards EGFR/ERBB2 heterodimers and an aggressive metastatic phenotype.

Aims

In basal-like/triple-negative breast cancer patients we will correlate clinical data with the abundance of ERBB receptors and evaluate ERBB receptor abundance and dimers during metastatic progression (Aim 1). Next, in basal-like cellular models, we will dissect the impact of low/high EGF administration on ERBB expression, EGFR/EGFR and EGFR/ERBB2 dimers activation, global gene expression and cellular phenotype related to tumor dissemination (Aim 2). Finally, we will evaluate the combinatorial administration of anti-HER2 drugs and high doses of EGF in a pre-clinical mouse model of basal-like cancer growth and dissemination (Aim 3).

Experimental design

In basal-like breast cancer patients, we will correlate clinical parameters and evaluate ERBBs abundance and dimers formation in primary tumors, lymph nodes and distant metastases.

By manipulation of EGFR and ERBB2 and in vitro administration of high/low EGF doses to basal-like cellular models and mammospheres derived from basal-like primary/metastatic lesions, we will assess the transcriptional cross-regulation between EGFR and ERBB2, and the impact of their homo/heterodimers on global gene signature, cell differentiation and anchorage-independent survival/growth.

The role of EGFR and ERBB2 in tumor dissemination will be assessed by genetic manipulation in a highly and poorly metastatic basal-like cancer mouse models in vivo. Finally, the administration of high EGF doses, alone or in combination with anti-HER2 drugs, will be assessed in vivo on tumor growth and dissemination in basal-like/triple-negative cancer mouse models.

Expected results

We expect that EGFR is reduced, while ERBB2 is upregulated during the metastatic progression of basal-like breast carcinoma. In primary tumors, EGFR/EGFR homodimers signaling would reduce ERBB2 expression and promote epithelial differentiation. The transition towards EGFR/ERBB2 heterodimers would promote a less-differentiated/mesenchymal metastatic phenotype. Thus, the administration of high doses of EGFR or anti-ERBB2 agents, alone or combined, may promote cell differentiation and inhibit tumor dissemination.

Impact on cancer

Currently, triple-negative/basal-like breast cancers patients have very limited treatment options and our project may suggest the administration of anti-HER2 agents or high EGF doses, or their combination, as novel differentiation strategies.

Alessandra Fiore

Università degli Studi di Verona, Verona

Mutant-p53 induces ferroptosis resistance in pancreatic cancer

Background

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal diseases in Western societies, with an average 5-year survival rate of 6%. PDAC resistance to conventional chemotherapeutics has been related to the complexity of this tumor type, where genetics and different microenvironmental factors interact. Untangle this complexity is mandatory to identify biomarkers and prognostic factors as well as new therapeutic approaches.

Hypothesis

We recently demonstrated that modulation of the tumor microenvironment (TME) can promote cancer progression by inducing anti-ferroptosis. Moreover, our preliminary data suggest that TP53 mutations, that are one of the most common genetic alterations in PDAC, induce resistance to ferroptosis by yet unknown mechanisms. Therefore, we hypothesize that mutations in the tumor suppressor p53 will induce an abnormal ferroptotic response that completely reprogram the TME thus promoting cancer progression.

Aims

The overall aim of this project is to assess the contribution of mutp53 in promoting PDAC progression by regulating anti-ferroptotic responses. We will systematically analyze the complex network of intracellular and secreted mutp53-dependent regulators of ferroptosis. We will use different in vivo PDAC mouse models to identify PDAC subtypes with different intrinsic sensitivity to ferroptosis and we will assess innovative therapeutic approaches. In parallel, we will screen sera of PDAC patients to identify new biomarkers of ferroptosis, starting with the putative target GPX3. Finally, we will improve current molecular classification of PDAC by integrating genetics and the novel ferroptosis-regulators network building up a risk model of response to therapy.

Experimental design

The project is articulated into three main distinct and complementary tasks. In the first task, we will set up in vitro and in vivo ferroptosis assays to investigate the contribution of mutp53 in regulating tumor progression, accompanied by a complete morphological analysis and time-resolved profiling at the transcriptional and translational level. In the second task we will analyze the mutp53-dependent extracellular component via metabolomics and patient sera proteomics in order to identify new biomarkers for PDAC. Finally, our results will be validated in a cohort of 150 cases of PDAC to identify novel determinants of PDAC subtyping and define novel strategy to stratify patients and predict patients' response to therapies.

Expected results

Successful implementation of the herein proposed aims will yield to the recognition of a novel ferroptosis-regulators network and the identification of novel biomarkers for PDAC progression and future drug targets.

Impact on cancer

Pancreatic cancer is a devastating disease with an increasing incidence and death rate worldwide. The

achievement of this project will bring new insight in the field of cancer research. Indeed, we expect to generate fundamental knowledge about the role of anti-ferroptosis in pancreatic cancer progression and resistance to conventional therapies. Moreover, the project is bound to have direct translational impact by identifying early PDAC biomarkers as well as novel therapeutic options based on the modulation of the TME.

Valentina Fragliasso

Azienda Unità Sanitaria Locale di Reggio Emilia IRCCS, Reggio Emilia

Preserving DNA: investigating the genomic role of HELLS in the development and progression of T-cell Lymphomas

Background

T-cell Lymphomas (TCLs) are a rare and heterogeneous group of non-Hodgkin lymphomas with aggressive courses and poor prognoses. Mechanisms underlying their progression are not fully elucidated limiting the development of targeted therapeutic strategies. A high transcriptional rate is required to support massive cancer cell proliferation but the transcription is a dangerous process that facilitates DNA damage and genomic instability. We recently identified HELLS -a ubiquitously expressed DNA-helicase deputed to DNA structure resolution- as a vulnerability of TLCs demonstrating that HELLS orchestrates a transcriptional program, impacting on survival of TCLs.

Hypothesis

We hypothesize that HELLS plays a bi-modal transcriptional function in TCLs. By binding a subset of immune-specific promoters, HELLS primes the accessibility of the genome to TFs coordinating immune-related transcriptional programs favoring RNAPII recruitment and transcriptional activation. Instead, by avoiding DNA-topological conflicts, this helicase facilitates RNAPII progression contributing to support high-rate transcription while supervising genomic stability.

Aims

The goals of this proposal are

1) shed light on the molecular mechanisms through which HELLS integrates transcription and DNA maintenance on cancer-specific sites supporting TCLs progression (AIM 1-2).

2) Identify and develop specific inhibitors to exploit the synthetic lethality between HELLS inhibition and drugs approved for the treatment of TCLs (AIM3).

Experimental design

Using the combination of "omics" (RNA-sequencing, ChIP-sequencing, DRIP-sequencing and DSP) and molecular functional approaches we will define: i) its cooperation network in controlling immune response, ii) the role of HELLS in genome integrity and iii) the mechanisms through which HELLS balances the transcription and DNA-repair. Performing CRISPR and synthetic lethality screenings, we will identify potential drugs with synergistic activity to HELLS depletion. Furthermore, we will identify and characterize selective anti-HELLS compounds by in silico approach followed by in vitro and in vivo functional experiments.

Expected results

Obtained results will help to elucidate the mechanisms through which HELLS sustains TCL progression. Furthermore, by identifying small molecules with anti-HELLS properties and evaluating their potential in reducing cell proliferation, we will provide proof of principle data to support the use of anti-HELLS compounds as anti-lymphoma agents.

Impact on cancer

Although important steps have been made in defining the pathobiological mechanisms of TCLs, many shadow areas and unresolved questions remain, leaving a significant portion of patients without the most adequate therapies. At the crossroads between replication, transcription, and DNA repair, HELLS is an attractive target for developing novel anti-cancer treatments for hematological malignancies. Dissecting the role of HELLS in promoting TCL progression, we will gain novel in-depth insights into the pathogenesis of TCLs. Integrating "omics" data with functional experiments, we will approach these diseases from a different and translational perspective generating new milestones in the biology and the treatment of TCLs, and candidating HELLS as a novel therapeutic target. Moreover, being HELLS deregulated in many tumors, the knowledge generated by this study will be relevant to other cancer models.

Irene Franco

Università Vita-Salute San Raffaele, Milano

Mechanisms of somatic mutation and tumor initiation downstream hypoxia-driven metabolic changes in pre-malignant kidney

Background

Understanding the genetic mechanisms that guide the evolution from a normal to a cancer cell may enable strategies of tumor prevention. In the kidney, the most common tumor type, named clear cell renal cell carcinoma (ccRCC), is derived from a specific cell subset. Somatic mutations that confer malignancy traits are progressively accumulated, starting from the first decade of life. The most common cancer-driver event in sporadic ccRCC is the disruption of the tumor suppressor gene and regulator of the response to hypoxia Von Hippel Lindau (VHL). When VHL mutations are inherited, they cause a tumor-predisposition syndrome characterized by a 70% lifetime risk of ccRCC.

Hypothesis

Our preliminary data show that the precursors of ccRCC accumulate somatic mutations faster than other kidney cell types and this excess of mutations is likely due to a rewiring of cellular metabolism in conditions of hypoxia (either due to micro-ischemic episodes or pseudo-hypoxia consequent to VHL-loss). These metabolic changes are amenable to pharmacological regulation. Therefore, we hypothesize that targeting specific metabolic pathways (e.g. conversion of glutamine into glutamate, excessive pyrimidine biosynthesis, production of the oncometabolite 2HG) may slow down mutation accumulation and tumor evolution in the kidney.

Aims

First, we plan to demonstrate the causal relationship between metabolic changes downstream hypoxia in pre-cancer kidney cells and somatic mutagenesis underlying ccRCC. Secondly, we aim to provide the proof of concept that pharmacological targeting of specific metabolic pathways can reduce somatic mutagenesis in pre-cancer cells and eventually reduce penetrance of ccRCC in predisposed kidneys.

Experimental design

The landscape of somatic mutations in pre-cancer and cancer genomes from the kidney of VHL patients and controls will be analyzed by exploiting my published method for somatic mutation detection in single genomes (WP1). The molecular mechanism leading to the observed mutation pattern will be elucidated by analyzing primary kidney cells (obtained from the urine) exposed to putative genotoxic conditions, such as hypoxia and metabolic alterations (WP2). The genotoxic effect of selected metabolic changes will also be assessed in vivo, in mice treated with hypoxia or diets that alter glutamine metabolism (WP3). Finally, specific strategies to reduce the impact of hypoxia-mediated metabolic changes on somatic mutation accumulation will be tested in primary kidney cells from VHL patients (WP4).

Expected results

We expect to dissect the molecular mechanism connecting kidney hypoxia, metabolic changes and loss of genome integrity and validate metabolic targets able to reduce somatic mutation accumulation and prevent

ccRCC development in the kidney.

Impact on cancer

While pharmacological targeting of metabolism is not a suitable strategy for kidney tumor prevention in the general population, this approach can be adopted for those patients affected by genetic diseases that induce predisposition to renal cell carcinoma, such as VHL and other inherited syndromes. These patients develop numerous kidney tumors that constitute the leading cause of death. Despite early diagnosis of the inherited disease and multiple decades required for ccRCC to develop, patients have no therapeutic options to reduce or delay tumor evolution. If proved effective, the presented strategy for tumor prevention will revolutionize the clinical management of VHL and other genetic diseases.

Giovanni Germano

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The impact of the non-coding genome on response to immune-based therapies in gastrointestinal cancers

Background

DNA mismatch repair (MMR) is central to maintaining genome fidelity during cell divisions. Alterations in MMR lead to microsatellite instability (MSI) and occur in multiple tumor types showing a marked and long-lasting response to immune checkpoint blockades (ICBs). We and others have demonstrated that inactivation of MMR increases the mutational burden and leads to dynamic and persistent renewal of neoantigens, which triggers robust immune responses. Whether and to what extent alterations in non-coding genomic regions affect tumor immune surveillance remain largely unknown.

Hypothesis

In this proposal, we hypothesize that an altered MMR repair complex contributes greatly to the alterations in the non-coding portions of the DNA in addition to the coding region. In particular, we postulate that specific regulatory elements such as the 5'untranslated regions (5'UTR) and long non-coding RNA (lncRNA) may exert an immune modulatory effect in cancer, since recent evidence confirms that these portions can encode peptides.

Aims

This research program aims to: i) characterize the fraction of tumor-derived antigens originating from coding and non-coding portions of the genome and study how these antigens are differentially perturbed by DNA repair deficiencies; ii) define the immune compartments triggered by non-coding DNA alterations and determine how they contribute to the antigenicity of tumor cells; iii) and functionally characterize how non-coding portions of DNA might shape the antigen profile and affect immune surveillance in gastrointestinal cancers.

Experimental design

We will take advantage of a large and fully annotated database of patient derived organoids and murine tumor cell lines carrying alterations in MMR genes. The analysis of RNA and whole-genome sequencing will confirm the characteristics of tumor alterations before and after in vivo growth. In addition, a peptidomic approach will allow us to move beyond prediction to the identification of tumor antigens. Furthermore, we will exploit gene editing methods to address the role of unexplored DNA portion in perturbing the response of gastrointestinal tumors to ICB.

Expected results

The project might reveal an unexplored role of non-coding DNA in the crosstalk between cancer cells and the immune system. Preliminary evidence in the proposal highlights the 5'UTR as the most highly edited part of the genome. Analysis of the genetic landscape of tumors after growth in immune competent mice prompted us to determine the non-coding DNA regions that mainly contribute to the immune editing. Furthermore, we expect to better characterize the 5'UTR and lncRNA sequences in terms of antigenicity and

correlation with the response to ICB.

Impact on cancer

The genetic landscape of tumors may have a severe impact on the immune response and we plan to understand how the non-coding part of the genome might instruct the immune system. Hence, we were prompted to define the manner in which the alterations across the entire genome of MMR deficient cells contribute to the favorable response to ICB therapy. The identification of non-coding regions that can trigger an immune response could suggest a predictive biomarker of response to ICB. In addition, the identification of peptides encoded by 5'UTRs and lncRNAs, that can provoke an immune response, could be pivotal for anticancer therapies.

Simona Giunta

Università degli Studi di Roma "La Sapienza", Roma

Investigating the basis of cancer and aging-associated centromere instability in human cells

Background

Cancer starts from a single catastrophic cellular event that affects genome stability and initiates malignant transformation. Centromere DNA repeats have been omitted from all current human whole genome cancer studies. There is a pressing need to integrate a new understanding of centromere sequences and function in cancer studies, especially in light of their essential role in faithful genome duplication at each round of cell division.

Hypothesis

I propose that centromere instability is a driving force behind cancer. I hypothesize that centromere DNA changes directly impact the process of cell division and my early evidence suggests that centromere repeats stability is maintained by a specialized DNA damage response and a unique checkpoint cascade that contribute to cancer suppression. These yet-unidentified players represent a novel class of tumor suppressor genes that work to promote chromosome integrity through maintenance of centromere DNA.

Aims

My overarching aim is to understand the mechanisms that contribute to centromere instability, how changes in centromere DNA translate into structural and functional disruption of chromosome dynamics, and systematically identify centromere causal variants in the human population associated with cancer. Specifically, I will:

(1) comprehensively identify factors operating to maintain centromere repeats stability; (2) uncover the molecular identity of the centromere-associated cell cycle arrest and the role of centromere DNA instability in the early stages of malignant transformation; (3) sequence centromere DNA to define the causal link between centromere heterogeneity, chromosome structural alterations and cancer susceptibility, and establish a new diagnostic tool for various tumor types.

Experimental design

The design comprises experimental and computational parts, as wells as the development of novel scientific approaches, including high-throughput CRISPR/Cas gain-of-function and loss-of-function screens integrated with bioinformatics tools. Genomic and proteomic approaches will decipher the centromere-specific proteome and unravel networks in combination with genetic perturbations. Genome-engineering will assess centromere disruption in relation to senescence, aneuploidy, and dysplasia. A novel approach combining long-reads sequencing and optical mapping will provide centromere sequences to use for population and cancer cohorts studies.

Expected results

The work funded by AIRC will yield ground-breaking information in: (1) understanding the role of centromere DNA in cancer; (2) identifying the centromere protection network, including novel tumor suppressor genes

and new checkpoint arrest to prevent carcinogenesis; (3) linking centromeric chromosomal features with errors in chromosome segregation and downstream mutagenic events; (4) a new approach to sequence human centromeres, identification of centromere-specific cancer driving mutations and biomarkers development.

Impact on cancer

Establishing centromere DNA as a cancer driver will be transformative in the way we think of cancer. Because centromere sequences vary across the human population and between individuals, cancer-linked centromere variants may represent a key contributor to cancer predisposition providing meaningful opportunities for the development of diagnostic tools for cancer susceptibility and early detection through liquid biopsies. Understanding how centromere DNA mutations promotes direct tumor formation will inform therapeutic interventions and cancer prognosis. Furthermore, uncovering the axis centromere instability - aging - cancer will substantiate the age-associated increase in cancer incidence. Finally, the centromere stability network will uncover new tumor suppressor genes and pathways to prevent cancer and maintain genome stability.

Giovanna Grimaldi

Consiglio Nazionale delle Ricerche, Napoli

Overcoming breast cancer resistance to hormone-based therapy by targeting the PARP12 enzyme

Background

Breast cancer is one of the leading types of cancer in women; in approximately 70% of total cases, proliferation and growth of breast cancer cells rely on estrogen receptor (ER) signaling, therefore hormone-based therapy is the standard of care for these tumors. Despite its high successful rate, still a substantial fraction of patients dies due to the acquisition of drug resistance.

ADP-ribosylation is a post-translation modification catalyzed by PARP enzymes that regulates essential cellular processes, often altered in diseases. PARP12 - a member of the family- has been associated to the onset of drug resistance in ER+ breast cancers, making this enzyme a promising drug target. The molecular basis underlying its involvement in the acquisition of resistance are unknow to date.

Hypothesis

The main hypothesis of the present proposal is that PARP12 contributes to promote survival of breast cancer cells, possibly by intervening in the PI3K/Akt/mTOR pathway, and hence counteracting PARP12 enzymatic activity may be beneficial in the treatment of ER+ breast cancers. Multiple biological mechanisms concur to the development of resistance, including mutation in the ESR1 gene, alterations of the PI3-Kinase/Akt/mTOR pathway or of the cyclin-dependent kinases 4/6 pathways, for which combinatorial therapies using targeted drugs have proven to ameliorate survival. Here, we hypothesize that the PARP12-mediated signaling is part of those pathways activated in non-responding patients, therefore targeting PARP12 is here presented as a novel breast cancer therapeutical strategy.

Aims

Our main aims are i) to dissect the molecular basis of the PARP12-mediated ADP-ribosylation pathways underlying cell survival of breast cancer cells; ii) to investigate the interplay between PARP12- and estrogen receptor-mediated signaling; iii) to identify drug-like PARP12 selective inhibitors for in vivo use as anticancer agents in breast cancer tumors.

Experimental design

The project is organized in 3 main tasks. First is the dissection of the PARP12-mediated signaling in breast cancer cell models by: a) analyzing the effects of PARP12-mediated ADP-ribosylation on Akt activation and functions and b) identifying the ADP-ribosyl proteome of PARP12 "sensitive" models. Second is the study of an interplay between PARP12 and ER□-dependent transcription, by analyzing the breast cancer transcriptome and the regulation of the receptor multiprotein complex activity. Third is the development of PARP12 specific inhibitors that impairs breast tumor growth.

Expected results

We expect to delineate the molecular basis of the PARP12-dependent cell survival in a subset of breast cancer tumors, and to develop PARP12 inhibitors to be proposed as novel drug, eventually for combinatorial

therapies.

Impact on cancer

To date, approximately 40% of endocrine-resistant breast tumors hold known genomic alterations, while the remaining 60% still rely on undiscovered alterations. According to statistics, breast cancer still represents the first cause of death in women. High levels of PARP12 in ER+ breast cancer correlate with a poor survival. With the execution of this project, we expect to provide the basis for the development of a novel treatment for breast cancer tumors relying on altered PARP12-driven pathways.

Paolo Grumati

Fondazione Telethon, Pozzuoli

Dissecting selective autophagy role in oesophageal and colorectal neoplasia development

Background

Cancer cells are exposed to stress elements that induce ER stress, which influences the survival of malignant cells. Cells activate the UPR to restore the ER homeostasis. Autophagy activation is a well-known biological response to ER stress and UPR. In cancer, autophagy acts as a bimodal process that can promote cancer development or induce cytotoxicity. Whether selective autophagy and in particular ER-phagy is involved in cancer is still not clear. In ESCC and CRC, pathological mutations in the ER-phagy receptor FAM134B have been described and associated to the biological aggressiveness of the tumors.

Hypothesis

Our hypothesis is that an altered ER-phagy is pathogenic for cancer development. ER-phagy is activated to resolve ER stress and has a dual role: allows cancer cells to adapt to the microenvironment, thus the oncogenic property of FAM134B in ESCC, or ii) has cytotoxic effect, and this will explain the tumor suppressor role of FAM134B in CRC. Unraveling the molecular mechanisms that regulate the pro- or anti-cancerogenic role of ER-phagy will be instrumental to develop alternative and targeted therapies.

Aims

1. Role of ER-phagy and macro-autophagy in cancer cells proliferation, survival and migration.
2. Biochemical characterization of FAM134B interactome and post-translational-modifications.
3. Characterization of the signaling pathways (de)regulated by ER-phagy in cancer cells.
4. In vivo pharmacological induction of ER-phagy to counteract ESCC.

Experimental design

For the first aim we will employ a genetic and a pharmacological screen to interrogate the autophagy machinery in CRC and ESCC cells and receive a feedback in terms of cell viability. For the genetic screen we will use multiplex gRNAs libraries against autophagy genes. Multiplexed reagents will allow to unravel the complexity of autophagy molecular signalling in cancer cells. For the pharmacological screen, we will treat cells with two libraries of chemically characterized compounds: i) autophagy modulators to affect cancer cell viability, ii) FDA approved drugs to perturbate ER-phagy.

For the second and third aim we will take advantage great potential of MS to provide an unbiased insight into the molecular mechanisms. We will analyze FAM134B interactome and PTMs as well as full proteomic profiles of our cancer cells. Ca2+ signaling will be monitored using the AEQ probes, while cell death and ER stress will be investigated using commercial reagents. Nude mice injected with Kyse-450 cells will be employed in the last aim.

Expected results

We expect to have a comprehensive over-view of the signalling pathways that regulate ER-phagy in CRC and ESCC including a detailed biochemical characterisation of FAM134B. From the genetic screen we will have

precise indications regarding the gene-gene interactions that regulate ER-phagy; while from the pharmacological screen we will highlight the chemicals suitable for the therapeutic modulation of ER-phagy.

Impact on cancer

The ending point is to improve the mechanistic understanding of the physiological role of ER-phagy in cancer progression to instruct new therapeutically strategies and drug design. Unraveling the role of ER-phagy in CRC and ESCC will be useful in the study of other cancers, where it is involved. Directly targeting ER-phagy, modulating FAM134B, could be an effective strategy to couple with chemotherapy.

Annamaria Gullà

Istituto di Candiolo - Fondazione del Piemonte per l'Oncologia (FPO) - I.R.C.C.S., Candiolo

Exploiting phagocytosis checkpoints as novel immunotherapeutic targets in multiple myeloma

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Background

Multiple myeloma (MM) is a plasma cell neoplasm that accounts for about 20 percent of deaths from hematologic malignancy and 2 percent of deaths from all cancers. A profound immune dysfunction features disease progression and hampers the clinical benefit of immunotherapeutic approaches. Hence, a deeper understanding of the mechanisms underlying MM immune escape may provide novel targets to therapeutically restore anti-tumor immune surveillance and prolong patient survival.

To this direction, we have recently demonstrated that dying tumor cells may provide immunogenic stimuli activating a dendritic cell (DC)-mediated immune response that culminate in tumor clearance by endogenous T cells. This immunogenic cell death modality can be triggered by clinically active compounds, such as proteasome inhibitors, and it is dependent on the exposure by dying tumor cells of "eat me" signals, such as calreticulin. In further preliminary work, we identified a novel "don't eat me" phagocytosis checkpoint axis which can hamper MM clearance.

Hypothesis

We hypothesize that inhibitory phagocytosis checkpoints are aberrantly expressed in MM driving tumor immune evasion. As a corollary to this hypothesis, we propose the targeting of inhibitory phagocytosis checkpoints as a novel therapeutic strategy to restore sensitivity to immunotherapeutic drugs.

Aims

In this proposal we will define the landscape of inhibitory phagocytosis checkpoints in the MM tumor microenvironment (Sp. Aim 1). We will leverage our preliminary data to functionally characterize a novel inhibitory phagocytosis checkpoint axis (Sp. Aim 2). Finally, we will optimize strategies for the therapeutic targeting of the inhibitory phagocytosis axis in clinically relevant models of MM (Sp. Aim 3).

Experimental design

We will integrate available transcriptomic data of clinically annotated MM patients with surface detection of inhibitory phagocytosis checkpoints in MM and antigen presenting cells to define the landscape of inhibitory phagocytosis checkpoints and its impact on MM immune evasion (Sp. Aim 1). We will then avail of well established in vitro and in vivo models of MM to genetically manipulate the expression of inhibitory phagocytosis checkpoints and validate their role in the suppression of MM cell phagocytosis (Sp. Aim 2). Finally, we will exploit our validated screening platform to optimize clinically applicable antisense oligonucleotides targeting a selected inhibitory phagocytosis checkpoint. These antisense molecules will be tested in our clinically relevant pre-clinical models in vitro and in vivo, for future translation to clinical trials alone or in scientifically-informed combination with immune therapies (Sp. Aim3).

Expected results

Our work will define the landscape of inhibitory phagocytosis checkpoints in MM and characterize the role

of phagocytosis dysfunction in tumor immune evasion, response to immunotherapy and poor survival in MM patients. We anticipate to validate the role of a novel inhibitory phagocytosis axis in MM, susceptible of therapeutic intervention using a clinically applicable RNA medicine approach.

Impact on cancer

This proposal will provide a novel understanding of the biological and therapeutic significance of inhibitory phagocytosis checkpoints in cancer. Our studies will establish phagocytosis checkpoints as a novel immune target for translation to clinical trials in the MM setting.

Nereo Kalebic

Fondazione Human Technopole, Milano

Role of the morphology of glioblastoma stem cells in proliferation and invasiveness

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Background

Glioblastoma (GBM) is a major unmet clinical need, showing severe resistance to therapy and a median survival of 15 months. The poor outcome of the therapy is linked to a striking molecular heterogeneity among and within GBM and the infiltrative phenotype, which remains an unresolved biological question. GBM stem cells (GSCs) are thought to be a key cell type underlying the proliferative capacity and invasiveness of GBM.

Hypothesis

This study is based on the hypothesis that the morphology of GSC plays an underlying role in the GSC proliferation and invasiveness. It builds on my postdoctoral findings that the morphology of neural progenitor cells during brain development plays a key role in their proliferation and cell behaviors. As these cells show a striking resemblance to GSCs, we are translating this concept to tackle the cell biology of GSCs.

Aims

In this project, we aim at identifying and characterizing the morphological features of GSCs and linking them with the cell's molecular signature. We further aim at identifying morphoregulatory genes and the molecular mechanisms underlying GSC proliferation and invasiveness.

Experimental design

Through our clinical collaboration, we will obtain IDH-wt GBM samples after surgery and generate patient-derived GBM organoids from them. Building on our preliminary data, we will analyze the morphological features of GSCs in organoids employing advanced microscopy techniques. Additionally, we will tackle the molecular heterogeneity of GSCs in organoids via scRNA-seq. Subsequently, we will link the morphological and molecular features and examine the proliferative and invasiveness capacity of different morphotypes. We will further perform a high-throughput CRISPR-based perturbation of selected and prioritized morphoregulatory genes in GSCs, which will be followed by a robust readout of invasiveness and proliferation performed in three hierarchical steps, with the easily screenable assays performed first. Most promising genes will be followed up in a 3D co-culture system of GBM and cerebral organoids, which mimics the in vivo scenario of infiltration into healthy tissue. Lastly, an in-depth characterization employing genomics, microscopy, proteomics and xenografting approaches will be performed on the top targets.

Expected results

We will identify and molecularly characterize those GSC morphotypes that show the greatest proliferation and invasiveness. We will further identify those morphoregulatory genes that are underlying the GBM proliferation and invasiveness. Finally, our in-depth characterization of selected morphoregulatory genes will elucidate the mechanisms underlying GSC proliferation and invasiveness.

Impact on cancer

Identification of GSC morphoregulatory genes as potential drug targets will pave the road to future drug screens, that can have a direct impact on developing targeted therapies to block the GBM invasiveness.

Federica Laudisi

Università degli Studi di Roma "Tor Vergata", Roma

Dissecting the antineoplastic role of rafoxanide in colorectal cancer

Background

Colorectal cancer (CRC) is still one of the leading causes of cancer-related deaths worldwide, mainly due to the lack of effective treatment of advanced disease. Despite major efforts aimed at discovering and validating novel and effective drugs against CRC, the process of developing such agents is often protracted, exacting and costly. Drug repositioning, defined as the use of existing drugs for new therapeutic indications, has been gaining popularity in oncological drug development as it provides the opportunity to expedite promising anti-cancer agents into clinical trials.

Hypothesis

The anthelmintic agent rafoxanide (RFX) is approved by the Food and Drug Administration for the veterinary treatment of some gastrointestinal nematodes. We recently found RFX inhibited proliferation in CRC cells, but not in normal colonic epithelial cells, in vitro and in a mouse model mimicking human sporadic CRC. These effects associated with the induction of endoplasmic reticulum (ER) stress and immunogenic cell death (ICD), a particular form of apoptosis able to elicit anti-tumor immune responses. Such preliminary observations indicated RFX as a promising anti-cancer agent in CRC.

Aims

The proposed research program aims at assessing a) the mechanisms underlying the RFX-driven ER stress and ICD, b) whether and how RFX may affect the CRC immune microenvironment and/or other cancer-related hallmarks (i.e., microbiota and cell metabolism), c) whether and how RFX may inhibit/restrain the development of different CRC subtypes (e.g., colitis-associated, serrated) as well as the progression of advanced disease and the spread of metastases.

Experimental design

To achieve the indicated goals, we will employ a broad approach consisting of a) conventional cellular and molecular biology techniques (e.g., western blotting, flow-cytometry) and siRNA- and/or CRISPR/CAS9-based strategies aimed at the selective knock-down/overexpression of molecules involved in RFX-affected pathways, b) CRC patient-derived tumor infiltrating leukocytes, biopsies/specimens and organoids, c) unbiased OMICS-based strategies (e.g., transcriptome analysis, proteomic/phospho-proteomic analyses, 16S rRNA sequencing and metabolome analysis), d) conventional, transgenic and state-of-the-art animal colorectal tumor models recapitulating many of the features of human CRC progression, including even spontaneous metastases to the liver and lungs.

Expected results

Our exploratory observations suggest that RFX may negatively affect major CRC hallmarks. We expect to determine a) the basic mechanism/s by which RFX induces ER stress and ICD in CRC cells, b) the effects of RFX on CRC-related tumor immunity, gut bacterial populations and related metabolites, c) the role of RFX in the modulation of cancer/immune/stromal cell metabolism in CRC, d) the effect of RFX on the in vivo

initiation/growth of colonic tumors and the spread of metastases. We anticipate that our results will provide significant insights into the anti-neoplastic functions of RFX in CRC as well as clues on whether the drug may represent a suitable therapeutic option for improving CRC treatment and patient outcome.

Impact on cancer

This study will have translational implications and potential clinical relevance as it will help determine whether RFX, either alone or in combination with other therapeutics, could be deployed as an anti-cancer drug in patients (or in selected patient populations) affected by CRC.

Alessandra Lugo

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Cancer incidence and mortality attributable to cigarette smoking in Italy:risks and impact of tobacco control strategies

Background

More than one out of three cancer diagnosis worldwide could be avoided by modifying preventable risk factors, including tobacco. Cigarette smoking is highly associated with at least 15 cancer sites, including lung, oral cavity, larynx, stomach, kidney and bladder. Also, exposure to second-hand smoke (SHS) increases the risk of selected respiratory-tract cancers among non-smokers.

Hypothesis

It is important to monitor the trend in smoking habits and SHS exposure according to the change of tobacco control measures at national level, to investigate the support to these legislations by the population, and to evaluate and compare the effectiveness of tobacco control measures on the burden of smoking-related cancers. Moreover, data on dose-response association between smoking/SHS exposure and the risk of cancer are missing, although crucial to provide accurate estimates of the cancer burden due to smoking, both at individual and population level.

Aims

The main aim of this project is to provide a comprehensive picture of the cancer burden attributable to smoking in Italy by: i) providing updated and accurate quantification of the association between cigarette smoking/SHS exposure and the risk of tobacco-related neoplasms; ii) quantifying the number of deaths and DALYs caused by these risk factors in Italy; iii) evaluating the effectiveness of different preventive strategies in reducing the burden of smoking-related cancers in Italy.

Experimental design

Cancer-specific systematic reviews and meta-analyses on dose-response associations between smoking/SHS exposure and the risk of tobacco-related neoplasms will be conducted using an innovative methodology (WP1). Population attributable fraction (PAF) from smoking/SHS exposure will be computed with different methodologies using relative risks derived from WP1, individual-level and aggregated data on smoking habits from national surveys, and number of cancer deaths and DALYs in Italy (WP2). A cross-sectional study will be conducted to obtain key indicators on smoking to be compared before and after the implementation of tobacco control policies in Italy (WP3). Simulation models will compare the effectiveness of preventive strategies in reducing the burden of smoking-related cancers in Italy (WP3). A dedicated website will be populated with the project findings, including a living systematic review to keep updated the main results of the meta-analyses (WP4).

Expected results

This project will provide the most up-to-date and accurate quantification of the effect of cigarette smoking and SHS exposure on cancer risks, investigating for the first time the dose-response relationships with selected smoking-related variables. The number of deaths and DALYs that could be prevented by eliminating

cigarette smoking/SHS exposure in Italy will be obtained and a new methodology to estimate PAF will be developed and implemented. A user-friendly tool will allow users to interact with the website to obtain yearly estimates of cancer-specific deaths and DALYs attributable to smoking for the current levels of exposure or under different prevention scenarios.

Impact on cancer

Project findings will have relevant implications on a cancer prevention perspective, providing useful information to guide policy decisions aimed at controlling tobacco smoking. Moreover, these data could represent the baseline for an update of the IARC monograph on tobacco smoking and cancer risk, and could be integrated in the GBD estimates.

Federica Marasca

Fondazione "Istituto Nazionale Genetica Molecolare - INGM", Milano

Reprogramming exhausted T cells through the disruption of chromatin condensates

Background

Immune Checkpoints Inhibitors (ICIs) have symbolized an unprecedent progress in cancer therapy; however, most of the patients fail to achieve a successful response. One of the reasons is the establishment of a population of terminal exhausted T cell (Tex) that are insensible to ICIs treatment. Hence, the study of the epigenetic mechanisms steering the acquisition of a terminal exhausted phenotype is pivotal to design novel and more effective strategy to reprogram Tex into functional effector cells (Teff). At present, it is acknowledged that chromatin organizes into condensates to control gene expression; interestingly, defects in condensate assembly are associated to a plethora of diseases, representing an innovative window for drug discovery.

Here I present a solid bunch of evidence proving that in exhausted T cells heterochromatin is assembled in aberrant condensates, characterized by a marked increase of H3K9me3 foci. Interestingly, the interference with chromatin condensates drifts back Tex to a more functional phenotype.

Hypothesis

Here I postulate that T cell exhaustion is a "condensatopathy" as regulated by the aberrant assembly of heterochromatin condensates. I hypothesize that the interference with heterochromatin condensates could represents a new way to revert T cell exhaustion.

Aims

This proposal aims at illuminating how heterochromatin condensates steer terminal T cell exhaustion, a knowledge that is still missing in tumor infiltrating lymphocytes (TIL) biology. Furthermore, it aspires to demonstrate that targeting heterochromatin condensates in TIL could represent a novel, still yet unexplored frontier to define novel druggable molecules for cancer treatment.

Experimental design

I aim to study how heterochromatin condensates regulate the genome during the transition of Teff into precursor and terminal Tex. To this aim, I will define i) genes regulated by heterochromatin condensates by ChIP-seq for H3K9me3, Kap1 and HP1a and ii) their local proximity by Hi-ChIP for H3K9me3. Also, I will iii) inspect the topology of the identified heterochromatin condensates by DNA-FISH combined with H3K9me3 immunostaining in super resolution microscopy.

In parallel, I aim to identify which proteins compose and regulate the dysfunctional assembly of heterochromatin condensates in terminal Tex i) performing an optical CRISPR/Cas9 screening that tests H3K9me3 foci assembly by imaging. Finally, I plan to interfere with the assembly of the aberrant heterochromatin condensates to revert terminal T cell exhaustion ii) targeting the identified proteins with aptamers able to perturb the propensity of the molecules to phase separate. This approach has been designed to increasing the specificity of "epigenetic-based therapies", as it avoids to deplete the cells of

essential epigenetic regulators and/or enzymatic activities.

Expected results

This proposal promises to define new T-cell biology, discovering uncharted epigenetic mechanisms and to identify novel candidate targets and molecules for immunotherapeutic approaches in the promising field of "condensates-modifying therapeutics".

Impact on cancer

The produced results will go towards precision and increasingly specific medicine and could renovate immunotherapy approaches by targeting the "inner" of the cells instead of the "outer", namely awakening the effector response of TIL by drugging "chromatin condensates".

Ionica Masgras

Consiglio Nazionale delle Ricerche, Padova

Taming the metabolism of tumor associated macrophages to fight peripheral nerve neoplasms

Background

The metabolic rheostat of tumor cells is often altered, thus posing a strong constraint in the tumor microenvironment and possibly shaping its immunological response. Despite a remarkable macrophage infiltration in malignant peripheral nerve sheath tumors (MPNSTs), Schwann cell (SC) cancers hardly hitting patients with Neurofibromatosis type 1 (NF1) genetic disease, the role of innate immunity is poorly understood. Tumor Associated macrophages (TAMs) are emerging as important players in the tumor microenvironment sustaining several pro-neoplastic and immunosuppressive activities. Recently, the diverse macrophage phenotypes have been found tightly connected to specific metabolic signatures pointing to the expression of certain metabolic enzymes as reliable markers of TAM specific functions. Narrowing down this information to key metabolic routes that sustain TAM pro-tumoral signals in MPNSTs could reveal novel therapeutic opportunities for reverting macrophage mis-behavior to the benefits of anti-neoplastic cures.

Hypothesis

Building on the metabolic adaptations discovered by our group and others in MPNST cells, we envisage that the metabolic status of tumor SCs, beside regulating tumor growth in a cell-autonomous manner, affects neighboring cells driving a pro-tumoral macrophage response. Uncovering specific TAM phenotypic/metabolic signature(s) in MPNSTs could bring space for novel anti-tumoral interventions based on macrophage re-education that could elicit an immune response effective in hampering MPNST growth.

Aims

The overall ambition of this proposal is to 1) Identify immune(metabolic) markers in MPNST samples from mouse models and human specimens, 2) Discover the metabolic-driven signals of MPNST cells that short-circuit pro-tumoral macrophage activities and 3) Re-educate the metabolism of tumoral macrophages to repress MPNST growth.

Experimental design

Exploiting our newly developed mouse model of MPNSTs we will characterize the phenotypic/metabolic features of macrophages infiltrating peripheral nerve neoplasms, thus defining a TAM metabolic signature. This will be subsequently tested in human MPNST specimens. We will combine transcriptomic and metabolomic analyses to pinpoint crucial metabolic routes through which tumor SCs signal to and engage neighboring macrophage metabolism for supporting cancer growth. A set of metabolic enzymes distinctive of MPNST-associated macrophages will be pharmacologically and genetically targeted assessing their anti-neoplastic efficacy in vitro and in vivo in immunocompetent MPNST-bearing mice.

Expected results

Merging macrophage markers of murine models and human MPNST specimens, we expect to identify key

metabolic enzymes underlying TAM pro-tumoral functions in MPNSTs. Their targeting is expected to affect in vitro/vivo MPNST tumorigenicity and/or to improve the efficacy of immune checkpoint drugs, thus leading to translational applicability.

Impact on cancer

This proposal points to the identification and targeting of Achilles' heel in TAM metabolism to achieve anti-neoplastic goals against currently incurable MPNSTs. A successful outcome of these investigations will reveal not only unprecedented targetable actors in non-tumoral cells of MPNSTs but empower also the discovery of the paracrine signals released by MPNST cells to hijack macrophage behavior. Unraveling the immunological blockchains occurring in MPNSTs could amplify the number of targeted therapies available for these aggressive cancers creating possibilities for synergistic interventions.

Domenico Mattoscio

Università degli Studi "Gabriele D'Annunzio" Chieti Pescara, Chieti

Resolvins as Novel Therapeutics to Enhance Anti-Tumor Immunity

Background

In the recent years, progresses in therapies through multidisciplinary care, in particular with the introduction of immunotherapies, lead to an improvement in clinical outcomes in head and neck cancers (HNC). However, only a small subset of patients benefits from these therapies, indicating that innovative approaches to stimulate anti-tumor immunity are urgently required.

Hypothesis

Accumulating evidence suggests that cancer-associated non-resolving inflammation is a major hurdle to overcome for successful immune response, since it prompts an immunosuppressive tumor microenvironment that hampers anti-cancer immunity. Thus, central hypothesis of the proposed research is that stimulating resolution of inflammation can represent a safe, effective and innovative strategy to boost host immune responses against tumors.

Aims

The present proposal pursues to dissect the role of a family of pro-resolving mediators, the D-series resolvins (RvD), as boosters of anti-cancer immunity. To achieve this goal, the activities of the project are organized in three work packages (WP).

1) To define RvD anti-cancer actions in pre-clinical models of HNC
2) To dissect the immune-modulatory mechanisms of anti-tumoral RvD
3) To evaluate RvD anti-cancer potential in humanized systems

Experimental design

The ability of the full range RvD (1 through 5) in reducing tumor growth will be tested in syngeneic tumor-transplanted mice and in mouse models of oral-induced carcinogenesis recapitulating both HPV+ and HPV- HNC subgroups. Immunohistochemistry and bulk RNA sequencing of tumor cells will be used to define how the selected anti-cancer RvD dampen tumor development (WP1). Furthermore, single-cell RNA sequencing of tumor-infiltrated leukocytes coupled with multiplex cytokine analysis and profiling of inflammatory lipid mediators in tumors will provide a detailed characterization of how selected RvD modulate anti-tumor immunity also in combination with current pharmacological treatments (WP2). Finally, the anti-cancer and immune-modulatory value of selected RvD will be confirmed in humanized HNC mice with single-cell RNA sequencing of tumor-infiltrated leukocytes, and by evaluation of cancer hallmarks (proliferation, apoptosis, production of mediators with tumor potential) in patient-derived tumor fragments (WP3)

Expected results

By means of a unique combination of advanced experimental approaches and in vivo models of disease, we foresee that RvD (likely RvD3 and D5, as determined in ongoing work) may prove beneficial by 1) reducing tumor growth; 2) promoting an immune stimulatory environment with increased infiltration of cytotoxic vs suppressive cells; 3) reprogramming selected leukocyte activities against cancer; 4) dampening cancer-

related inflammation, through an improved balance of pro-resolving vs pro-inflammatory mediators production

Impact on cancer

This innovative, translational, preclinical research will define roles and mechanisms of action of RvD as booster of anti-tumor immune response in HNC. This is of paramount importance as, despite aggressive multi-modality treatment (surgery, radiotherapy, chemotherapy, targeted therapy) HNC considerably affect quality of life and survival of patients. Since RvD already proved safety and effectiveness in trials of inflammatory disease, results of this project will foster transitioning of RvD to the clinic as stand-alone or combination therapy in a relatively short time-frame. The proposed research will thus promote the development of innovative immune-stimulating treatments with the goal of improving HNC management.

Alessandra Metelli

Istituti Fisioterapici Ospitalieri (IFO), Roma

From Coagulation to Cancer Progression: Exploring Diverse Roles of Tissue Factor in Lung Cancer

Background

Tissue Factor (TF) is a hallmark of poor prognosis in 80% of human aggressive malignancies, therefore representing an appealing target for cancer immunotherapy. TF enhances tumor growth employing platelet-independent and platelet-dependent mechanisms. As a main mediator of coagulation, TF enhances platelet activation with consequent release of TGFbeta, which is a potent pro-tumorigenic biomolecule. Other than on cancer cells, TF is expressed on lymphocytes, however its function in the context of cancer immunotherapy is not known. Tisotumab vedotin, an anti-tissue factor (TF) drug conjugate shows antitumor efficacy, however it blocks TF hemostatic function, thus causing severe bleeding events. Therefore, there is an urgency to create safe therapies to block platelet derived TGFbeta on tumor cells, without altering systemic homeostasis.

Hypothesis

In line with my preliminary data and with the existent literature, TF enhances TGFβ signal transduction both in cancer cells, where it triggers their metastatic transformation, and T lymphocytes, where it drives Tregs differentiation and ablates T effector cells cytotoxic activity.

Aims

To address my hypothesis the following aims will be pursued:

1. Describe how TF modulates TGFβ signaling in cancer cells.
2. Evaluate TF immunomodulatory effects on T lymphocytes.
3. Evaluate the safety and efficacy of the non-anticoagulant TF/TGFBR2 TRAP antibody for cancer therapy.

The rationale of the proposed aims is that unveiling how TF augments TGFβ signal gives me the mechanistic-driven knowledge to design local antibody-based which block pro-tumorigenic TF functions both in cancer cells and T cells

Experimental design

Aim1: The role of TF/FVIIa complex in TGFBR1 expression and activation will be evaluated by generating CRISPR TF KO cell lines. Also the physical association of TF with TGFBR1/TGFBR2 upon stimulation with TGFbeta or FVIIa will be tested. Bulk-RNA sequencing and protein profiling and phosphorylation status will be evaluated in WT and TF-KO cells stimulated with TGFbeta or FVIIa or in presence of platelet-rich plasma.

Aim2:CD4cre TFfloxed and CD8cre TFfloxed mice will be created. Aging experiments, tumor experiments, and tumor immunotherapy experiments will be conducted. Additionally, the contribution of platelets will be evaluated using platelet blocking antibodies in vivo.

To evaluate the clinical translation, tumor biopsies from NSCLC patients have been collected. Lymphocytes will be isolated and sorted based on the expression of TF, then single-cell RNA sequencing will be performed.

Aim3:Generation and selection of anti-TF/TGFBR2 TRAP. Pharmacokinetic, in vivo bio-distribution, safety and

anti-tumor property will be tested in human TF Knock-in mice.

Expected results

Upon completion of my aims, the expected outcomes are 1) to have defined a new role for TF in TGFβ/Smad signal transduction; 2) to have demonstrated the existence and the function of TF on CD4+ T cells; 3) to have proposed the anti-TF/TGFBR2 TRAP as an efficacious and safe therapeutic agent for cancer patients.

Impact on cancer

The proposed study is designed to acquire mechanistic knowledge of the platelet-dependent and platelet-independent TF role in cancer. This dual function of TF will be dissected both on cancer cells and on T lymphocytes to guide me in the generation of a bispecific antibody that, while maintaining homeostasis, is efficacious in cancer patients.

Rosanna Mezzapelle

Università Vita-Salute San Raffaele, Milano

Role of tumor microenvironment in CXCR4-mediated antitumor immunity

Background

Chronic inflammation and the presence of an unfavorable inflammatory tumor microenvironment (TME) can promote tumor development. A typical example is malignant mesothelioma (MM), an aggressive and incurable tumor arising from the exposure to asbestos fibres. High Mobility Group Box 1 protein (HMGB1), a non-histone DNA chaperone that also works as a signal of danger, contributes to MM onset and progression.

We recently discovered that CXCR4 (a G protein coupled receptor) activation by BoxA, an antagonist of HMGB1, causes tumor cells recognition by immune system in a mechanism involving macrophage phagocytosis. We named this mechanism Immunogenic surrender (IGS).

Hypothesis

Our hypothesis is that macrophages are essential for the mode of action of BoxA in IGS, and that untangling the structural determinants of BoxA/CXCR4 interaction is fundamental for exploiting IGS as a therapeutic intervention. We also envision that IGS could favorably synergize with checkpoint inhibitors therapy.

Our preliminary data indicate that macrophages are the main infiltrating cells in MM and that they support MM growth. We found that BoxA binds to CXCR4 with high affinity and that shorter variants of BoxA are still effective in inducing IGS in vitro. Moreover, BoxA not only makes tumor cells more visible to the immune system, it also reduces the expression of PD-L1, a negative check-point

Aims

We propose to 1) investigate the role of macrophages (MOs) in inflammation-related tumor growth and in IGS; 2) structurally and functionally understand the BoxA/CXCR4 interaction; 3) test if IGS synergizes with checkpoint inhibitors.

Experimental design

In Aim 1 we will characterize the macrophagic infiltrate in in vitro 3D models of mesothelioma and we will study the effect of BoxA on macrophage phenotype. We will also (task 1.2) investigate in vivo the involvement of MOs in tumor growth and in IGS.

In Aim2 we will structurally and functionally characterize the interaction between BoxA and CXCR4 and we will develop new molecules that retain BoxA efficacy but that will be easily translate to clinic.

In Aim3 we will test in vivo the multimodal treatment of BoxA with check point inhibitors in a model of MM.

Expected results

We expect to elucidate the role of macrophages in mesothelioma growth and in IGS mechanism, to identify small analogs of BoxA that trigger IGS and provide preclinical data about the combined treatment of BoxA with check point inhibitors.

Impact on cancer

We believe that the findings derived from this project may be applicable not only for the cure of

mesothelioma but for the entire field of the immunotherapy. We figure that reaching these goals will have a significant impact in complementing the available cancer therapy toolbox.

Marco Montagner

Università degli Studi di Padova, Padova

Dissecting genetic heterogeneity and microenvironmental cues for lung colonization of disseminated breast cancer cells

Background

Metastases are the major cause of death for patients with solid cancers and yet, effective therapies targeting or preventing metastases are missing. Several types of cancers, like estrogen-receptor positive breast cancer, show delayed relapses, with metastases occurring years after removal of primary tumor. This offers a therapeutic window so far unexploited. Clinical and experimental data showed that metastasis is an extremely inefficient process and only a minority of heterogeneous disseminated cancer cells are endowed with aggressive traits, but we have been unable to identify and characterise them so far. Whether metastatic cancer cells are dangerous or harmless is dictated by cell-intrinsic mechanisms as well as by the crosstalk with the microenvironment. Recently, we showed that lung-disseminated breast cancer cells activate TFEB-lysosomal axis, but its role in the survival and growth of metastatic cancer cells is still unclear.

Hypothesis

By isolating monoclonal populations of breast cancer cells with increasing metastatic proclivities it will be possible to identify genes/pathways/processes required for the different steps of metastatic cascade with unprecedented resolution. Our hypothesis is that TFEB-lysosomal axis sustains survival of metastatic cells at different stages of dissemination. TFEB-lysosomal axis might play a crucial role in the adaptation to the new microenvironment by several mechanisms, such as crosstalk with other organelles, regulation of other signalling pathways and metabolic rewiring.

Aims

1) Isolating clonal prospective metastatic breast cancer cells with increasing aggressiveness
2) Understanding the role of TFEB-lysosomal axis in survival, dissemination and relapse of metastatic cells
3) Identifying pathways required at different stages of metastasis
4) Discovering of new biomarkers for the identification of aggressive circulating tumor cells
5) Characterization of lung parenchymal response to dissemination

Experimental design

We will exploit CaTCH technique to identify and isolate prospective metastatic breast cancer cells with increasing metastatic proclivity. Lung parenchymal response to dissemination will be studied with multispectral imaging of lung tissues and recapitulated in a lung organotypic system. This system will be utilized to analyze in detail lysosomal accumulation, vesicle trafficking, as well as TFEB signalling network in the metastatic clones. Single cell RNAseq and whole genome sequencing will be used to study TFEB-driven and additional transcriptional programs at the basis of circulation, seeding and relapse of breast cancer cells in human and mouse preclinical models. Genetic profiling will be used to identify new biomarkers that will be experimentally validated prior to preliminary assessment of their clinical validity.

Expected results

The focus on specific clones will allow us to uncover new mechanisms, so far overlooked, underlying specific steps of the metastatic cascade. The thorough characterization of TFEB-lysosomal axis and of the lung parenchymal responses will shed light on the adaptation strategies developed by disseminated cancer cells. The analysis of circulating tumor cells with and without disseminating potential will provide candidate biomarkers for the identification of dangerous clones.

Impact on cancer

By identifying survival mechanisms and biomarkers specific for disseminating and relapsing metastatic breast cancer cells, we will be able to design new therapies and diagnostic strategies targeting the dangerous clones of metastatic breast cancer cells, with the final goal of blocking metastatic recurrences.

Eugenio Morelli

Istituto di Candiolo - Fondazione del Piemonte per l'Oncologia (FPO) - I.R.C.C.S., Candiolo

Decoding the molecular pathways and therapeutic potential of long noncoding RNAs in multiple myeloma

Background

Multiple myeloma (MM) is a malignancy of plasma cells that accounts for ~20% of deaths from hematologic cancers. More effective therapies are urgently awaited and may be informed by better defining the landscape of actionable tumor-promoting factors. Whereas most research focuses on proteins, there is emerging interest in the tumor-promoting activity of long non-protein coding RNAs (lncRNAs).

LncRNAs are key regulators of cellular function, acting either as gene regulatory elements or RNA transcripts. In this latter case, they are defined by having a length >200nt and lacking protein-coding potential. These RNA molecules outnumber protein-coding mRNAs in the human genome and are transcribed by dedicated promoters in a tissue-specific manner. A key function of lncRNAs is to provide molecular scaffolds for proteins and other nucleic acids. By this and other mechanisms, lncRNAs are becoming increasingly implicated in the progressive gain of a malignant phenotype by tumor cells.

In prior work, we identified a large number of differentially expressed lncRNAs in patient-derived MM cells versus healthy donor-derived plasma cells and found that lncRNAs are independent risk predictors for clinical outcome. We have started to functionally delineate the tumor-promoting activity of lncRNAs and demonstrated the actionability of two of them in animal models of MM (lnc-17-92 and MALAT1). Building on these early results, in this project we aim to better understand the tumor-promoting roles of lncRNAs and to unlock their therapeutic potential in MM.

Hypothesis

We hypothesize that lncRNAs control key survival pathways in MM cells and can be targeted for therapeutic benefit in MM-bearing animal models.

Aims

We will systematically define the tumor-promoting lncRNAs in MM and their clinical and functional features (AIM 1). Then, we will focus on a tumor-promoting lncRNA (RMRP) that has unique clinical, functional, and structural properties and that we hypothesize to be druggable for therapeutic effect. We will characterize its tumor-promoting mechanism (AIM 2) and develop small molecule and antisense inhibitors for its targeting in pre-clinical models of MM (AIM 3).

Experimental design

We will exploit the RNA-targeting endonuclease CRISPR-Cas13d to identify the tumor-promoting lncRNAs in an unbiased genome-wide manner and will couple it with single-cell RNA-seq (PERTURB-seq) to systematically define the transcriptional programs associated with the perturbations of the tumor-promoting lncRNAs (AIM 1). We will use RNA-protein interaction assays and live-cell microscopy to dissect the molecular interaction and functional role of a novel tumor-promoting lncRNA that we hypothesize provides an essential scaffold for the formation of nucleoli (AIM 2). Finally, we will optimize RNA-targeting

small molecule and antisense inhibitors of this lncRNA and test their anti-MM activity in cellular and animal models (AIM 3).

Expected results

We will define the tumor-promoting lncRNAs in MM and develop one of them as a therapeutic target with inhibitors for translation.

Impact on cancer

This project will unlock a novel repertoire of actionable targets and develop an effective way to target them. Our data will be a key asset to the MM and lncRNA fields and accessible on a public portal.

Ilaria Muller

Fondazione I.R.C.C.S. Ca' Granda Ospedale Maggiore Policlinico, Milano

Cross-reactive immune responses between breast cancer and thyroid autoimmunity: impact on prognosis and treatment

Background

Patients with breast cancer (BC) seem to have a higher prevalence of thyroid autoimmunity (TA), and this association has a solid pathogenic rationale: 1) I have discovered that BC tissue expresses thyroid peroxidase (TPO), a major thyroid autoantigen which exists in different isoforms by alternative splicing; 2) the immune system has a key role in both autoimmunity and cancer; 3) immunity and gut microbiota are deeply connected. Serum anti-TPO autoantibodies (TPOAb), the hallmark of TA, are highly prevalent among BC patients and they are associated with a better BC prognosis in retrospective studies. The magnitude of the relationship between BC and TA needs to be defined in longitudinal studies, unbiased by concurrent anti-cancer treatments.

Hypothesis

Patients with both BC and TA have a better BC prognosis, since they express intra-tumoral breast-specific TPO isoforms that are related with TPO-specific immune responses, and microbiota patterns, that contrast neoplastic progression, not present in patients affected with only BC and not associated TA.

Aims

1) To verify the prevalence and prognostic impact of TA in the first unbiased longitudinal cohort of BC patients, recruited before anti-cancer treatments; 2) To investigate breast-specific TPO isoforms expression in BC tissue; 3) to characterise TPO-specific tissue-resident immune responses in paired human samples derived from BC and thyroid-related tissues/organs; 4) to explore the gut microbiota associated with BC and TA.

Experimental design

Monocentric 5-year longitudinal study. BC patients and non-cancer controls will be sampled from paired blood, stool, tumoral-breast samples (BC only), and neck lymph nodes (LNs). LNs contain thyroid-draining lymphocytes and are easily accessible by ultrasound-guided fine-needle-aspiration, a well-tolerated technique which I routinely operate for research purposes. Methods used to address aims: 1) Serum thyroid function and TPOAb measurement to assess TA; 2) RT-PCR amplification and product sequencing of tumor-derived RNA in BC patients, to identify TPO isoforms expression; 3) Flow-cytometry immunophenotyping of TPO-specific lymphocytes; 4) Metagenomics, metabolomics and metaproteomics for microbiota analysis.

Expected results

BC patients have a higher prevalence of TA than non-cancer controls (Aim 1A). Compared with BC-only patients, those affected with both BC and TA have a better 5-year prognosis (Aim 1B), express breast-specific TPO isoforms (Aim 2), have increased tumor-infiltrating and neck-LN-resident TPO-specific lymphocytes with anti-neoplastic activity, i.e. T follicular helper cells (Tfh) (Aim 3), and show peculiar microbiota signatures (Aim 4).

Impact on cancer

This study will clarify the prognostic role of TA in BC patients by combining paired clinical data, intra-tumor antigen-expression and related lymphocytic infiltration, and microbiota patterns, generating novel clinical and therapeutic potential applications. Serum thyroid function and TPOAb, as well as intra-tumor TPO isoforms, may be assessed as routine BC markers. Precision immunotherapy for BC may target TPO isoforms; the benefits of improved tumor control would likely overweight the downside of thyroid adverse effects, easily manageable with levothyroxine replacement treatment. The immune cell subsets identified in this study may suggest other immunotherapies for BC. Finally, future clinical trials of probiotics, antibiotics, or fecal material transplant, may be designed to target the microbiota signatures of BC patients with and without TA.

Francesca Nazio

Università degli Studi di Roma "Tor Vergata", Roma

Dissecting the role of mitochondria in medulloblastoma stem cells dissemination and radiotherapy resistance

Background

Medulloblastoma (MB) is one of the most common paediatric cancers, accounting for 15-20% of all tumors of the central nervous system. Recent integrated genomic studies have classified MB into four different molecular (WNT, SHH, Group3, Group4) and clinical subgroups of which MBGroup3 is the one with the worst prognosis and usually display stem-like features.The standard treatment allows for an average survival rate of 60%, whereas the remaining 40% of patients still remains incurable. Various studies have shown that mitochondria play a central role in therapy resistance and relapse because of the ability of these organelles to modify cell metabolism, allowing survival and avoiding apoptosis clearance of cancer cells.

Hypothesis

To date, the role of mitochondrial regulatory mechanisms and their effects on the current therapeutic regimen and tumour dissemination in MB remain unknown as well as mitochondrial properties unique to MBSCs need to be defined. I hypothesize that differential physiology of mitochondria in MB stem and differentiated cells results into a different susceptibility to radiotherapy, suggesting that radiation-induced changes in MBSCs should have an effect on recurrence and metastasis post-radiotherapy.Moreover, our preliminary results suggest a key role for the mitophagy receptor NDP52 in regulating MBGroup3 cell survival, suggesting a possible mitophagy-dependent control of MBGroup3 metabolism.

Aims

Primed by the proven correlation that autophagy activation in MBSCs supports stemness and metastasization, and encouraged by our striking preliminary results we will develop an articulated multidisciplinary project plan to investigate:

A. the complex, context-dependent contributions of mitochondria in MBGroup3 aggressiveness;
B. the crosstalk among hypoxia, mitophagy and radiotherapy resistance in MB;
C. the pro-oncogenic role of the mitophagy receptor CALCOCO2 (NDP52) in MBGroup3;
D. how MBGroup3 maintains mtDNA integrity during tumour dissemination in vivo.

Experimental design

AIM 1: we will investigate radio-resistance-dependent
contributions of mitochondria functions and mitophagy, establishing radioresistant MBGroup3 clones compared to parental ones under both basal and hypoxic conditions

AIM 2: In order to explore the relevance of mitophagy players in MB stem cells, we will focus on the still underappreciated mitophagy receptor NDP52

AIM 3: We will use radiotherapy-adapted-(PDX) models of MB
recurrence to study whether radiation-induced changes in MBSCs should have an effect on recurrence and metastasis post-radiotherapy by acting on mitochondria-related mechanisms.

Expected results

After stressors such as RT, indeed, inducing severe DNA damage and
oxidative stress, MBSCs could use specific cytoprotective mechanisms (like the activation of mitophagy and mitochondrial stress) and changes in their metabolism to survive. Moreover, we expect that NDP52 play a crucial role in regulating MB aggressiveness. Finally, we expect that radiation-induced changes in MBSCs should have an effect on recurrence and metastasis post-radiotherapy by acting on mitochondria-related mechanisms.

Impact on cancer

Current MB therapies tend to kill the bulk tumour, rather than specifically target intrinsically resistant MBSCs that proliferate following completion of standard therapies. Moreover, leptomeningeal metastases are detectable in approximately 50-80% of patients at relapse and the mechanisms that drive MB dissemination have received less attention than other prognostic aspects. There is an urgent clinical need to develop new and more efficient therapies for the treatment of patients with high-risk MB.

Carlotta Palumbo

Università degli Studi del Piemonte Orientale "Amedeo Avogadro", Novara

Development of artificial intelligence-based multiplex network for individualized risk stratification of prostate cancer

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Background

Prostate cancer represents the most common neoplasia among men in Western countries, having a substantial impact on patients' lives and on healthcare systems. Its wide range of clinical aggressiveness requires an accurate disease risk stratification to tailor the treatments. Current classification tools' accuracy is still sub-optimal, especially considering low- to intermediate-risk patients, potentially resulting in over- and under-treatment. To date, single-biomarker or oligo-genic approaches have not shown a substantial predictive benefit and are not part of the routine practice. A promising prognostic and predictive role of artificial intelligence (AI) - based integrated platforms has emerged for other malignancies (notably, the ARIADNE platform for the triple-negative breast cancer profiling). To date, the role of these approaches in prostate cancer management is unexplored.

Hypothesis

We hypothesize that an artificial intelligence-based platform, integrating clinical, pathologic, imaging, genomic and transcriptomic profile of prostate cancer would outperform currently available risk-stratification tools, leading to a better definition of cancer progression and recurrence risk.

Aims

To define and develop a novel, sustainable and quantitative patient-based tool for risk profiling of prostate cancer, integrating genomic, transcriptomic, pathological, MRI and clinical data; to validate this predictive tool in a prospective multi-centre cohort of patients; to evaluate the predictive added value of a blood- and urine-based "liquid biopsy".

Experimental design

In the context of a multidisciplinary team of urologists and digital health experts, a two-phases study has been designed. A retrospective cohort of 200 radical prostatectomy patients will be identified within the three participating clinical centres. Clinical, pathology, MRI data will be collected and stored in an appropriate anonymised online platform. Whole exome sequences (DNAseq) will be analyzed for each patients (total samples=200) and transcriptome analyses (RNAseq) for both cancer and non-cancer tissues (total samples=400). In parallel, the recruitment of a prospective cohort of 200 biopsy-proven newly PCa patients will start. For these patients, blood and urine samples will be also collected. Data will be collected and genetic analyses (total samples=1,000) will be performed as in the retrospective phase. Patients will be treated and followed according to best clinical practice. All data will be analysed using an integrative complex systems approach and correlated with clinical outcome, thanks to the expertise of the Center for Complexity and Biosystems of the University of Milan. MRI and histology images will be segmented to extract quantitative features; genomic and transcriptomic sequencing will be analysed to identify mutation patterns, studying key pathways in depth.

Expected results

The retrospective phase would allow to identify genes, pathological features and MRI imaging features that can correlate with PCa biology, in order to create and train the AI-based algorithm. The prospective phase will allow the validation of the prognostic tool, the definition of a novel risk grouping and the evaluation of the prognostic role of biofluid analysis.

Impact on cancer

We anticipate that our risk-stratification tool will allow a better tailoring of treatments and patient counselling. In particular, identifying the true low-risk patients will enable a safer choice of active surveillance, saving potential over-treatment, while recommending radical therapy options to patients with an established risk of disease progression.

Sandro Pasquali

Fondazione I.R.C.C.S. Istituto Nazionale dei Tumori - Milano, Milano

Dissecting immunological effects of neoadjuvant therapies in primary high-risk soft tissue sarcomas

Background

Patients with high-risk soft tissue sarcomas (STS) at low survival probabilities are treated with neoadjuvant anthracycline and ifosfamide (AI) to lower their risk of developing metastatic disease after surgery. However, roughly one in two patients eventually recur mostly at distant site with limited therapeutic opportunities.

Hypothesis

Changes to immune infiltrate after neoadjuvant chemotherapy may effectively result in an immune induction that sensitizes tumours to an immune checkpoint inhibitor (ICI).

Aims

This study proposal is meant to demonstrate an immune modulation in primary localized high-risk STS of extremity or trunk wall after neoadjuvant chemotherapy with AI. Ultimately, these findings will provide evidence for a rationally designed and feasible randomised clinical trial (RCT) that will investigate an ICI in these patients. The amount of data generated to reach this main aim of this study will be exploited to identify whether an in-depth characterisation of the immune infiltrate could enhance the prognostic accuracy of patient risk stratification performed with the nomogram Sarculator, a widely diffused prognostic tool for STS.

Experimental design

Firstly, we will deeply investigate tumour immune infiltrate in samples of high-risk STS with the five most common histologies of extremity or trunk wall (undifferentiated pleomorphic sarcoma, synovial sarcoma, leiomyosarcoma, malignant peripheral nerve sheath tumour, myxoid liposarcoma) obtained before and after neoadjuvant AI. We will exploit multiple approaches, including the search for tertiary lymphoid structures (TLS), multiplexed IHC, and spatial transcriptomic analysis (WP-1). In order to monitor markers of tumour response and immunomodulatory effects of neoadjuvant AI at the systemic level, we will collect patient peripheral blood at three time points (i.e., diagnosis, after AI, after surgery) and test the presence of circulating tumour DNA (ctDNA) as well as the frequency and activation of myeloid, T-cells and B-cells (WP-2). All these data will be exploited to enhance the accuracy of Sarculator (WP-3). In parallel, we will validate a prognostic score, which was previously generated and is part of the preliminary data that support this study proposal. To validate findings from WP-1 and test effectiveness of ICI following neoadjuvant AI we will exploit patient-derived explants (PDE) of treated and untreated tumour samples (WP-4). Finally, we will rationally design a RCT for high-risk STS to be conducted within the Italian Sarcoma Group, the Italian network for sarcoma research and treatment (WP-5).

Expected results

We expect to identify changes induced by neoadjuvant AI in tumour cells, tumour-infiltrating and circulating immune cells in the most common high-risk STS of extremity or trunk wall. We also expect to improve current tools for patient staging.

Impact on cancer

This study will impact the field of cancer in five year times through generating compelling preclinical evidence through profiling immune modulation of AI in patients with selected high-risk STS both at the tumour and systemic levels.

Simone Patergnani

Università degli Studi di Ferrara, Ferrara

Relevance of mitochondrial HMGB1 for malignant pleural mesothelioma: from the neoplastic transformation to the therapy

Background

Pleural mesothelioma (PM) is an aggressive cancer highly correlated with exposure to asbestos. Once inhaled asbestos provokes a chronic damage to the mesothelial surface, causing malignant transformation. However, it remains unknown the precise mechanism(s) by which asbestos drives the initiation of the cell transformation process.

A deeper understanding of the molecular means involved in the onset and progression of PM is imperative to unveil targets for new therapeutic strategies.

Hypothesis

Several discoveries suggest that high mobility group box-1 (HMGB1) is a critical mediator of asbestos-induced PM initiation.

However, the HMGB1-related mechanisms regulating the malignant transformation are still unknown.

We hypothesize that, during the asbestos-induced cell transformation, HMGB1 is released from the nucleus to localize on the mitochondrial surface.

Here, HMGB1 induces mitochondrial dysfunctions and variations in ferritinophagy (a particular form of autophagy important for several cellular functions) and exacerbates the inflammatory environment, through the regulation of inflammasomes.

Aims

AIM.1: Investigate how dynamic changes of HMGB1 provoke mitochondrial dysfunctions and alter the iron-related mechanisms to regulate the chronic inflammatory state necessary to drive the malignant transformation.

AIM.2: Demonstrate that the asbestos-driven transformation is dependent on a mitochondrial localization of HMGB1.

AIM.3: Enforcing the anti-neoplastic properties of PM chemotherapeutic agents through strategies targeting the harmful HMGB1-mediated events.

Experimental design

The intracellular localization of HMGB1 as well as elements of the mitochondrial functioning, ferritinophagy and inflammasome will be monitored during the malignant transformation of primary human mesothelial cells and in mice exposed to asbestos using intrapleural injection. Variations of these intracellular dynamics will be also measured in tissue samples obtained from PM patients. Next, a series of HMGB1 chimeras inducing HMGB1 expression in different intracellular compartments will be designed and transduced in primary cultures obtained from conditional HMGB1 knockout mice to clarify the localization of HMGB1 that is important for exert these effects. Finally, the most promising compounds modulating the harmful HMGB1-mediated events identified in vitro will be tested in two murine models of PM to demonstrate that by

targeting these molecular pathways it is possible to counteract the PM progression and recurrence.

Expected results

We plan to unveil new molecular mechanisms of the involvement of HMGB1 in the evolution of the PM, in particular a specific molecular liaison composed of mitochondria, ferritinophagy and inflammasome. Further, the project will permit to understand whether monitoring HMGB1-related molecules may represent new tools to improve the diagnosis.

Finally, the project aims to reveal novel molecular strategies to improve the efficacy of conventional chemotherapy against PM.

Impact on cancer

PM is an incurable form of cancer characterized by a median survival from diagnosis of only 1 year. No effective therapies exist for this devasting disease.

Furthermore, PM is characterized by a rapid recurrence after surgery, as it is impossible to remove and kill every cancer cell.

This proposal aims to dissect new molecular mechanisms which occur during the malignant transformation of a mesothelial cells.

We are confident that a successful completion of this project will provide critical information for development of future therapies for PM and (in future) other cancers.

Livia Perfetto

Università degli Studi di Roma "La Sapienza", Roma

Modelling cell communication in Pancreatic Cancer: A Systems Biology Approach to Personalized Treatments

Background

Pancreatic ductal adenocarcinoma (PDAC) is characterized by a tumor microenvironment where cancer-associated fibroblast (CAFs) and cancer cells cross-talk via signals that result from the activation/repression of cell-specific signaling cascades. These processes sustain tumor growth and result in the deposition of a dense fibrotic stroma that shields the tumor from therapeutic treatments. Patient-specific proteogenomic profiling of cancer biopsies have shed light on general molecular events underlying PDAC tumorigenesis and progression, but have failed to clarify the molecular mechanisms that are deregulated in individual patients.

Hypothesis

The premise behind this proposal is that, by integrating all the molecular evidence collected over recent years, it should be possible to computationally model the dynamics of the events underlying the cell cross-talks occurring during PDAC onset and development at patient-resolution level. This step is necessary toward the development of effective personalized treatments. I advance that networks of causal interactions assembled by collating literature-evidence can provide a functional framework to integrate and interpret multi-omics datasets and build patient-specific models that are mechanistic or predictive.

Aims

This proposal aims at modeling the impact of cell communication on PDAC tumorigenesis and cancer progression to accelerate the rational usage of proteogenomic data in clinical practice. Specifically, I aim to i) Provide understanding of the molecular mechanisms underlying microenvironment formation; ii) Identify prognostic biomarkers; iii) Contribute to accelerate the identification of personalized therapeutic targets; iV) Support clinical decision-making.

Experimental design

First, I will annotate literature-derived interactions that play a role in the mentioned processes. Next, I will exploit a tool that I recently developed (SignalingProfiler) to combine curated data with publicly-available proteogenomic profiling of four large cohorts of PDAC samples to derive patient-specific mechanistic models of these processes. I will use them to derive biomarkers and prognostic factors (by applying machine learning followed by in silico and ex vivo validation); and to build patient-specific multi-scale models of intra- and intercellular communication between CAFs and cancer cells. These models will be trained with public data and tested to predict novel druggable nodes whose modulation can revert the malignant phenotype. Finally, I will develop a resource to access the project results.

Expected results

The main deliverables of this proposal are: i) a comprehensive description of interactions underlying PDAC; ii) 500 patient-specific mechanistic models; iii) a panel of biomarkers and prognostic factors; iv) approx. 30 patient-specific multi-scale models of intra- and intercellular communication between CAFs and cancer cells;

v) a ranked list of patient-specific targettable nodes; vi) a publicly-available resource for a rationale usage of proteogenomic data in clinical practice.

Impact on cancer

With a 5-year survival rate below 10%, PDAC remains one of the deadliest cancers. The development of novel and effective treatments, relies on the dissection of the cross-talk that sustains and protects the tumor. My proposal aims at addressing this point by delivering computational tools to model the tumor development and response to genetic and chemical perturbations. The project is innovative and, if successful, will have an impact in our understanding of PDAC etiology, in our ability to make accurate prognosis and in the design of effective treatments.

Geny Piro

Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Roma

New immunotherapy vulnerability determinants to improve treatment of microsatellite stable colorectal cancer patients

Background

To date, colorectal cancer (CRC) is still ranking top three major life-threatening cancers. In the last decade, immunotherapy has revolutionized the treatment for many cancer types showing very good results also in CRC patients with mismatch-repair-deficiency (MMRd) and high microsatellite instability (MSI-H), whether it is neoadjuvant therapy for operable patients or first-line or multi-line therapy for advanced patients. However, MSI-H is seen only in 10-20% of patients with early CRC cancer and 3-5% of patients with metastatic disease, whereas the vast majority of patients present a microsatellite stable status (MSS). Interestingly, recent clinical trials showed a promising activity of immunotherapy also in a subpopulation of MSS colon cancer patients, previously considered as an immunotherapy refractory population at a whole. These findings pave the way to further investigate immunotherapy efficacy in a biomarker-selected subpopulation of MSS colon cancer. Accordingly, in our preliminary data we identified two specific cell signatures linked to anti-PD1 sensitivity in MSS colon cancers.

Hypothesis

We hypothesize that biomarker-driven selection of MSS colon cancers could allow to identify those patients who could benefit from immunotherapy treatment. Moreover, the targeting of the identified signatures could improve immunotherapy response in treatment refractory patients.

Aims

This proposal is designed to improve patient selection for immunotherapy treatment and to identify new molecular based strategies to increase immunotherapy vulnerability of MSS colon cancer allowing the development of more effective therapeutic approaches to convert "immune-cold" into an "immune hot" tumor.

Experimental design

Starting from the strong results obtained in our preliminary data and the possibility to confirm our findings in samples from clinical trial evaluating immunotherapy in MSS colon cancer patients, we plan:

- To modulate the identified tumor-related pathways in MSS immunotherapy refractory colon cancers by using our ex-vivo immunity-organoids interaction platform and in vivo fully immunocompetent MSS colon cancer syngeneic mouse models.
- To target the identified microenvironment-related pathways.
- To translate our preclinical findings in clinical trials with MSS colon cancer patients using both a retrospective and a prospective approach.

Expected results

With our proposal, we expect to provide molecular based rational for addressing patients to the most appropriate therapeutic management providing new tolls to guide treatment decision making process for

MSS colon cancer patients and boosting immune system against cancer.

Impact on cancer

This proposal will lead to considerable progress in colon cancer treatment providing new insights in the understanding of molecular mechanisms responsible of immunotherapy resistance and tumor-immune escape pathways activation. Most importantly, we will be able to disclose new molecular biomarkers to further stratify patients for better therapeutic strategies. Thus, the results of this study will reflect in a change of clinical practice, helping to predict patients likely to respond to immunotherapy from those who will not.

Alessandro Porchetta

Università degli Studi di Roma "Tor Vergata", Roma

Activity-based sensing tools and kits for real time monitoring of DNA repair enzymes and in vitro drug screening

Background

Cancer cells often show reduced DNA repair capacity and higher genetic instability. Also the efficacy of chemotherapy treatments is strongly influenced by cellular DNA repair capacity. Although our understanding of DNA repair mechanisms has grown rapidly, toolkit for studying DNA repair enzymes has lagged behind. Standard cell-based assays for monitoring repair activities are well-established technologies but they are also indirect, laborious, and with limited applicability for clinical applications.

Hypothesis

The development of activity-based sensing platforms for the monitoring of repair enzymes, and their translation into clinical settings, can pave the way for improved early cancer diagnosis and novel personalized therapies based on repair capacity assessments in patients. To achieve this objective, CRISPR-based technology appears particularly attractive because it can be easily harnessed to convert the readout to a point-of-care tool. CRISPR also demonstrated the capacity to work robustly using biological samples and clinical specimens, and showed potential for high throughput screening (HTS) applications.

Aims

The transformative goal of my research program is to develop a synthetic biology toolkit enabling real-time, activity-based monitoring of DNA glycosylases involved in base excision repair (BER) of base pairs containing 8-oxoG. My efforts will be mainly devoted to the development of an activity-based CRISPR platform for the real-time monitoring of MutY homolog DNA glycosylase (MUTYH). The platform will be further adapted to produce innovative HTS assays (CRISPR-HTS) of small molecule modulators of MUTYH activity.

Experimental design

I will design and optimize highly specific nucleic acid networks capable of transducing the specific glycosylase activity into downstream CRISPR-powered ultrasensitive detection. First, I will design different classes of damaged nucleic acids (Obj1) as synthetic substrates of DNA glycosylases. Second, the repaired nucleic acid product will be integrated into artificial nucleic acid networks able to convert repair activity into CRISPR activators (Obj2). Finally, activity-based CRISPR platforms will be developed to monitor repair activity in tumor cell lysates (Obj3), and will be further adapted for HTS of drug candidates. To validate the platform, cytosolic extracts of commercial WT and MUTYH (-/-) HeLa cells will be used. Mouse embryonic fibroblasts of MUTYH (-/-) mice and lymphoblastoid cell lines derived from MAP patients with biallelic MUTYH mutations will be finally tested.

Expected results

I expect to develop 1) one reliable CRISPR-based platforms for the activity-based monitoring in real-time of MUTYH in clinical specimens of patients affected by MUTYH-associated polyposis (MAP); i) orthogonal nucleic acid-based systems for multiplex and specific monitoring of OGG1 and MUTYH glycosylases in blood serum;

iii) the identification of at least one selective modulator of MUTYH activity using a CRISPR-HTS platform.

Impact on cancer

Synthetic biology tools for the real-time analysis of MUTYH activity can represent a new paradigm for 1) testing therapeutic hypothesis concerning MUTYH and fully explore the implications of specific variants in MAP and colorectal cancer; 2) diagnosing disease states at early stage; 3) clarifying the role of MUTYH as a potential therapeutic target, also through the identification of small molecule modulators of MUTYH activity; 4) developing novel HTS platforms taking advantage of CRISPR technology and its superior sensitivity and specificity for nucleic acid detection.

Valentina Proserpio

Università degli Studi di Torino, Torino

Dissecting the molecular basis of Women's Extramammary Paget Disease malignancy by high-resolution omics

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Background

Extramammary Paget Disease (EMPD) is a rare malignant intra-epidermal adenocarcinoma that displays a high recurrence rate following surgical excision (40%) and is often accompanied by underlying malignancy (10%-30%). If surgery is not an option, for invasive EMPD (~10%) the outcomes are generally poor for the lack of curative therapy. Data about female vulvar PD (VPD) are almost absent, because of the rarity of the disease itself, but also due to a strong male predominance in the Asian population (where most of the studies are conducted). Surgery is often extensive and includes total vulvectomy, thus severely impairing the patient's quality of life.

Hypothesis

The molecular knowledge on EMPD comes from studies conducted on almost exclusively male patients. To fill this gap and understand if the same or other deregulated cellular and molecular mechanisms are present, we will investigate the molecular and cellular basis of VPD in a unique collection of female VPD samples including non-invasive and invasive, recurrent and not recurrent, Imiquimod responders and not-responders. Being a rare disease, this analysis represents a crucial resource to investigate the molecular basis of invasion, recurrence and Imiquimod responsiveness in women. The most promising/deregulated mechanisms will be then functionally validated first in vitro and then in vivo, to identify new potential therapeutic targets and prognostic markers in invasive VPD.

Aims

This project aims at increasing our understanding of VPD in women at molecular and cellular level to identify new deregulated genes or pathways to improve diagnosis, monitoring and therapy of VPD. This project aims at the identification of new patients stratification strategies to avoid extensive and mutilating surgeries, whenever are therapies are likely to be efficient.

Experimental design

Perform RNA-seq on FFPE/fresh tumor and healthy matched skin samples. From freshly resected samples we will perform Single-Cell RNA-seq and we will generate stable cell lines from both cancer cells and healthy surrounding tissues. These cells will be instrumental for perturbation and validation experiments to identify deregulated pathways and genes in vitro. Best hits will be then validated in vivo.

Expected results

With this project we aim at the identification of new biomarkers for detecting patients at risk of recurrence and new biomarkers to divide patients in subgroups for selecting the best intervention therapy in individual patients (personalized medicine).

Moreover, we aim at the identification of putative new therapeutic targets for re-purposing studies, thus reducing the surgical, often mutilating, approach.

Impact on cancer

Patients with VPD typically require extensive and often multiple surgeries due to its high recurrence rate. Moreover, the surgical treatment results in mutilating resections, with considerable morbidity, substantially affecting the patients' quality of life. Therefore, the development of new treatment for VPD and the identification of stratification strategies are both extremely significant and of great interest. Filling the gap in knowledge about VPD and defining the molecular mechanisms at the basis of VPD will help the clinicians in directing the therapies to limit the surgical approach.

Alberto Puliafito

Istituto di Candiolo - Fondazione del Piemonte per l'Oncologia (FPO) - I.R.C.C.S., Candiolo

Imaging treatment-driven population dynamics in colon cancer organoids

Background

Understanding how resistance to anti-cancer therapies emerges and how to overcome it is among the most important challenges of cancer research. Minor pre-existing tumor populations carrying specific genetic mutations or newly emerging populations harboring de-novo mutations are known mechanisms of resistance. However resistance to therapy can also emerge due to heterogeneities in the transcriptional status of cells rather than in the genetic makeup. Such scenario is relevant in those tumors that contain differentiation hierarchies, such as colorectal cancer (CRC).

In metastatic CRC that do not carry mutations in RAS, anti-EGFR therapies show tumor regression and decreased proliferative potential, but leave behind a residual disease (RD) that in the long term leads to resistance. It has been shown that differentiation hierarchies in CRC do have a role in response to therapy, and that phenotypic features of RD are significantly different from the original tumor.

Hypothesis

We put forward the hypothesis that CRC responders to anti-EGFR therapies are composed of different transcriptionally-defined cell populations that are related by differentiation hierarchies. Understanding the interactions between the different populations. i.e. asymmetric cell divisions, selective apoptotic rates, feedback mechanisms regulating the relative abundance will not only explain the observed phenotypic change in treated samples, but will also unveil potential access points to newly exposed vulnerabilities that might optimize combination therapies.

Aims

We aim at characterizing the transcriptional profile of existing populations in a set of CRC organoids that respond to Cetuximab and to characterize the changes in population structure and transcriptional traits. Lineage-tracing experiments will unveil the interactions between the populations. Quantitative modeling of populations will elucidate the common traits across different samples, and these will be aimed at identifing the population that is best targeting to reduce or eliminate the RD.

Experimental design

We propose to exploit (i) CRC organoids as realistic tumor models that are amenable to single-cell level live observations; (ii) single-cell RNAseq technology to describe populations in untreated and treated organoids; (iii) imaging-based lineage-tracing experiments that will allow to track single cells belonging to different subpopulations and their fates in terms of proliferation/apoptotic rates and differentiation into other subtypes; (iv) mathematical population models to integrate all the experimental data into a comprehensive falsifiable model that will define common underlying principles to therapeutic response of responder CRC samples.

Expected results

We expect to find the roots of the therapeutic response of CRC organoids to Cetuximab in the underlying

population dynamics and to explain mechanistically the interactions between the populations and their effect on the response to therapy. Such insights will be used to devise a rational combination treatment that focuses on the critical differentiation hierarchies steps rather than on overall mass reduction with the aim of tackling the MRD.

Impact on cancer

The current proposal addresses a fundamental cancer research question with several attachment points to therapeutic implications, notably the therapeutic relevance of the biological model, the presence of multiple patient-derived samples conferring a significance to the final results, the insights into the response to a clinically approved therapy (Cetuximab) and the investigation of novel targets to target the RD.

Linda Quatrini

Ospedale Pediatrico Bambino Gesù, Roma

Impact of corticosteroids on ILC reconstitution and function upon HSC transplantation to cure hematologic malignancies

Background

High-risk hematological malignancies currently represent the main indications for allogenic hematopoietic stem cell transplantation (HSCT). HSCT recipients are often treated with corticosteroids (GCs) for the prophylaxis and treatment of inflammatory reactions. It was shown that this treatment favours HSC engraftment, but whether it affects the amount, function and type of immune cell subsets generated on the short and long term is not known. We have recently set up an in vitro system for generating NK cells and hILCs from HSCs, which allowed us to demonstrate that GCs inhibit HSCs differentiation towards a common ILC precursor. Importantly, our preliminary data show that a 20h-exposure to GCs in culture is sufficient to cause the same impairment in HSC long-term differentiation ability towards ILCs, observed upon longer treatment conditions.

Hypothesis

These data led to the hypothesis that GCs are responsible for "training" HSCs, thus permanently affecting their developmental program towards ILCs. Upon HSCT, ILC deficiency may contribute to a defect in the control of disease relapse, lead to permanent alteration of tissue homeostasis and repair, and favour inflammation-induced carcinogenesis.

Aims

The aim of this project is to identify the HSCs gene expression modifications induced by GC treatment and its role on the reconstitution of circulating and tissue-resident ILC subsets upon HSCT, including both cytotoxic NK cells and hILCs. Importantly, we aim to understand whether it has a beneficial or detrimental role for the patient, not only in terms of HSCT outcome, but also for the long-term homeostasis of the mucosal tissues.

Experimental design

To address this aim we will perform in vitro experiments to study the molecular mechanisms responsible for GC regulation of HSC differentiation. We will validate the role of GC targets by HSC gene editing. We will also study HSC differentiation and the effects of GCs on ILC development and function in vivo, by transplantation of human HSC in immunodeficient mice. Finally, we will analyse the impact of GC treatment on the reconstitution of circulating ILCs and other immune cell subsets ex vivo in pediatric patients undergoing HSCT, and we will correlate the efficiency of this reconstitution to HSCT outcome.

Expected results

We will determine the intrinsic effects of GCs on the amount and function of immune cell subsets generated from HSCs. We will also shed light on HSC transcriptional and epigenetic regulation by GCs, identifying target(s) responsible for their effects on ILC development. In addition, we will characterize the reconstitution of circulating and tissue-resident ILC subsets upon HSCT and associate it to GC administration. In the end,

we expect to understand the effect of GC treatment on hemopoiesis.

Impact on cancer

The results of this study will have an impact not only for HSCT recipients, but in general on the management with GCs of oncologic patients needing hematopoietic recovery, for example following radio or chemo therapy. These patients would benefit from optimizing the doses and timing of GCs administration, to obtain the desired immune-suppressive effect, while taking into account their previously unappreciated impact on immune reconstitution.

Francesco Raimondi

Scuola Normale Superiore - Pisa, Pisa

Decoding and recoding onco-GPCR signaling through integrative bioinformatics and protein engineering

Background

As the resolution of omics approaches is increasing, there is a dire need to develop computational models able to provide mechanistic insights about the molecular principles governing the interaction between cancer cells and the tumor micro-environment (TME) which ultimately lead to cancer progression and therapy-resistance.

Hypothesis

Our fundamental hypothesis is that G-protein Coupled Receptors (GPCRs), the most important family of transmembrane protein transducers, play an important, yet largely unappreciated role in mediating interaction between cancer cells and TME. An overarching understanding through computational models of how GPCRs mediate intra- and inter-cellular signaling, can ultimately lead to define readily available cancer prevention strategies or targeted and immune therapies by targeting GPCRs either individually or in combinations with other drugs.

Aims

We aim to: 1) expand through bioinformatics the repertoire of GPCR signaling network mechanisms; 2) use such signaling networks to computationally interrogate high-dimensional, high-resolution omics datasets expected to shed new light on the role of GPCRs in cell-cell communication in cancer and suggest viable drug opportunities; 3) leverage signaling mechanism knowledge to engineer a new GNA13-Designer Receptor Exclusively Activated by Designer Drugs (DREADD)

Experimental design

We will improve predictors of G-protein and β-arrestin binding by integrating through bioinformatics and machine learning approaches new experimental datasets provided from collaborators or available in the literature. We will integrate the intracellular interaction network with curated information of extracellular ligands and metabolizing enzymes from available databases. We will use this network to interrogate high-resolution datasets either from public repositories or collaborators, e.g. single cell RNAseq (scRNAseq) of syngeneic mouse models of different cancer types, RNAseq and proteomics analysis coupled to Laser Micro Dissection of PDAC mouse models and human patients, and transcriptomics, metabolomics and metagenomics profiling of CRC and lung cancer patients. Bioinformatics analysis will entail state-of-art machine learning techniques for omics datasets integration as well as more advanced approaches, including new pipelines that we will develop to study intercellular crosstalk. On the other hand, we will exploit the determinants of GPCR signaling selectivity to design a new GNA13-DREADD. We will employ both a data-driven design approach and an in-vitro molecular evolution (ME) strategy, by leveraging engineered systems for ME in mammalian cells and using GNA13-selective GPCRs (e.g. GPR55) to seed the experiments.

Expected results

Our GPCR interaction network will constitute one of the most exhaustive resource to study GPCR signaling. This will illuminate the molecular basis of intercellular crosstalk in high resolution onco-omics datasets, and suggest new GPCR-based therapeutic options. Using our combined approach, we will obtain highly selective GNA13-DREADD and reveal minimum determinants of coupling selectivity.

Impact on cancer

These bioinformatics tools will ease the interpretation of omics datasets and, together with larger epidemiological cohort, will suggest via webapps novel therapeutic options based on approved GPCR drugs repurposing or better stratification strategies for immunotherapies. We also expect that our study will illuminate the molecular determinants of activation and selectivity of GNA13-coupling and will moreover provide chemogenetic tools to dissect in the future the poorly characterized G12/13 pathway of relevance in B-cell lymphomas and metastasis.

Francesca Reggiani

Azienda Unità Sanitaria Locale di Reggio Emilia IRCCS, Reggio Emilia

Wiring Natural Killer transcriptional network to prime anti-tumor immunity in Triple-Negative Breast Cancer treatment

Background

Triple-Negative Breast Cancer (TNBC) patients not responding to neoadjuvant chemotherapy (NACT) account for 60-70% of all cases and are orphans of therapeutic alternatives. Recently, the KEYNOTE522 study paved the way for the use of immunotherapy in neoadjuvant settings, obtaining a significant improvement in patient response. The implementation of chemotherapy with immunotherapy in the TNBC neoadjuvant treatment implicates several compelling questions on how to further increase the percentage of patients with a complete response. In this scenario, the stimulation of Natural Killer (NK) activation is a promising strategy. Still, a comprehensive characterization of intracellular and extra-cellular mechanisms governing NK activation toward TNBC is largely lacking or mainly addressed to tumor-intrinsic mechanisms. This issue further restrains the possibilities of intervention to properly stimulate NK activation and limits their clinical applicability.

Hypothesis

The central hypothesis of this project is that the activation of NKs can be stimulated by the modulation of key Transcription Factors (TFs) and that the resulting transcriptional reprogrammed NKs can improve TNBC patient response to the KEYNOTE522 neoadjuvant setting.

Aims

Our aims will be: 1) To fully characterize the transcriptional networks dependent on our candidate TFs (VEZF1, RXRA, and MAZ) that control NK activation toward TNBC; 2) To explore how transcriptional reprogrammed NKs, obtained by the modulation of our TFs, interact with other immune cells in the tumor microenvironment (TME); 3) To validate the therapeutic efficacy of adoptive NK cell therapies, by applying our transcriptional reprogrammed NKs in combination with the KEYNOTE522 neoadjuvant setting in ex-vivo and in vivo models.

Experimental design

We will integrate functional genomics and high-throughput approaches (RNA-sequencing, ChIP-sequencing) to decipher the transcriptional landscape of candidate TFs and how they control NK activation. A CRISPR-based approach will be applied to modulate the TF expression and generate transcriptional reprogrammed NKs. These cells will be characterized by functional analyses applying the state-of-the-art approaches in the study of TME (organoids, digital spatial profiling, multi-parameter flow cytometry, 3D imaging, scRNA-Seq). The ability of these adoptive reprogrammed NKs in enhancing neoadjuvant response will be then tested in ex vivo bioreactors and in vivo mouse models.

Expected results

We will characterize the transcriptional networks controlled by the candidate TFs (MAZ, VEZF1, and RXRA), clarifying their unknown role in NK activation. According to these results, we expect to select the top-scoring

TF whose modulation will be exploited to obtain transcriptional reprogrammed NKs. The impact of this reprogramming will be established on NK anti-tumor functions, as well as on the synergic interaction with other TME cells, and on the improvement of neoadjuvant response.

Impact on cancer

The contribution of this project will be significant to clarify how to exploit adoptive NK cell therapies in the treatment of TNBC patients that do not fully respond to the KEYNOTE522 neoadjuvant setting and if NKs can be used as biomarkers of response. Overall, this project can improve our understanding of NK anti-tumor functions and foster their application in the clinical management of this tumor. Besides, the obtained findings on NK biology can be translated to other tumors, opening novel opportunities in the immune-oncology field, beyond breast cancer.

Francesca Ricci

Istituto di Ricerche Farmacologiche "Mario Negri" I.R.C.C.S., Milano

Exploiting tumor metabolic vulnerabilities to delay and overcome the resistance to therapy in ovarian cancer

Background

The development of therapeutic strategies overcoming the resistance to platinum-based chemotherapy is an unmet medical need in ovarian cancer. Indeed, the onset of resistance to therapy represents one of the major causes of a poor prognosis.

Cancer metabolism has become of great interest as an hallmark of tumours. In particular, metformin (an anti-diabetic drug and a metabolism modulator) has been shown to have an antitumor effect. Moreover, the combination of particular diet regimens (precision nutrition) with a pharmacological treatment (precision medicine) is emerging as an effective approach for cancer treatment with reported increase response to therapy also in chemotherapy resistant tumors.

Hypothesis

Our preliminary data suggest that targeting the metabolic remodeling of platinum resistant patient-derived xenografts (PDXs) represents a promising therapeutic strategy, as the addition of metformin (MET) to cisplatin (DDP) is able to counteract the resistance (testing schedule). Interestingly, the combination of DDP and MET was also able to delay the development of resistance itself in a first-line experimental setting (preventing schedule). Then, we have corroborated that the addition of intermittent fasting with DDP and MET is well tolerated and able to increase the antitumor activity of DDP+MET therapy. Starting from these preliminary data, the proposal purses the hypothesis that the development of DDP resistance in ovarian cancer can be delayed and overcome by targeting metabolic adaptations of resistant tumours.

Aims

To address our hypothesis, the specific aims of the present proposal are:

1. understanding the impact of the combination of different diet regimens and the DDP+MET treatment in the delay/reversion of the DDP-acquired resistance in PDXs and syngeneic ovarian cancer models;
2. dissecting the intratumor heterogeneity at the basis of DDP resistance;
3. test/ validation of new therapies in preclinical models of ovarian cancer (PDXs and syngeneic).

Experimental design

The project will include the following Working Packages:

1) Overcoming DDP resistance by a "food-based" approach. The addition of intermittent fasting and methionine restriction with pharmacological therapy (DDP+MET) will be tested in ovarian cancer PDXs, and the role of immune-infiltrate in drug response will be evaluated in syngeneic ovarian cancer models.

2) Single-cell sequencing and spatial omics will be performed in residual and in the regrowing tumors after DDP challenge. These analyses will identify new pathways to be targeted to overcome/block the resistance to DDP.

3) Testing of the best therapeutic combination strategies in immunodeficient and immunocompetent

models of ovarian cancer. We will test both the ability to overcome an already acquired-resistance (testing schedule), and to delay the onset of the resistance itself (preventing schedule).

Expected results

This project will elucidate the mechanisms at the basis of the onset of DDP resistance in ovarian cancer, and the identification of new potential therapeutic strategies (diet and/or drug-based) to overcome/delay the resistance itself.

Impact on cancer

This project will lay the groundwork for a rapid translation of metformin and/or a particular diet regimen, a safe and cost-effective approach, with chemotherapy in the clinic, and the development of pilot clinical studies that exploit metabolic vulnerabilities to overcome platinum resistance both in the adjuvant/ first-line and in the relapsing resistant setting.

Patrizia Romani

Università degli Studi di Padova, Padova

A trade-off between ferroptosis and migration limits RAC1-dependent metastatic dissemination of melanoma cells

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Background

Despite recent advancements in the therapy against melanoma, metastatic dissemination remains a primary cause of patient mortality, and a clinically-unmet problem. During invasion, melanomas can switch from mesenchymal to amoeboid migration modes. Then, to disseminate through the blood vessels, melanomas must withstand ferroptosis lipid peroxidation cell death. Aggressive melanoma cells show a dedifferentiated phenotype and develop resistance to targeted therapies. However, metastatic aggressive melanoma cells are also intrinsically more sensitive to ferroptosis. What are the underlying molecular mechanisms and whether this represents a possible weak spot of melanoma remains unknown.

Hypothesis

We recently found that RAC1, a main factor instructing melanoma therapy-resistance and invasive ability, also induces cell sensitivity to ferroptosis. Activation of RAC1 promotes ferroptosis sensitivity by lowering the cells' antioxidant metabolism, and this regulation is intertwined, at the molecular level via IQGAP1 and KEAP1, with the ability of RAC1 to promote mesenchymal migration. This indicates the interesting possibility that ferroptosis and oxidative stress sensitivity represents a cost that melanoma cells need to "pay" to become invasive and migratory. This trade-off does not become evident until invading cancer cells disseminate through the blood, when ferroptosis kills RAC1-activated cells. This could explain why RAC1-activated melanomas are locally invasive, but poorly metastatic. This also indicates that acquisition of amoeboid migration, characterized by low RAC1 activity, and thus resistant to ferroptosis, represents a way by which melanoma cells retain migratory ability, but at the same time evade blood-induced ferroptosis.

Aims

With this grant we propose: (i) to identify what is the cause of blood-induced FPT; (ii) to better understand the regulatory logic and molecular mechanisms underlying RAC1-induced FPT sensitivity; (iii) to broaden its significance within melanoma subtypes; (iv) to explore how melanomas evade blood-induced FPT, including the mesenchymal-amoeboid plasticity; (v) to test whether inhibition of parallel pro-oncogenic RAC1 effector pathways (SRF and YAP/TAZ) can synergize with FPT induction, and whether it is possible to resensitize ameboid escaper cells to FPT.

Experimental design

We will study established models of human metastatic melanoma in vitro, and validate the significance for metastasis in mice. We will use hypothesis-driven and middle-size unbiased screens to shed new light on this process. We will perform gene expression, metabolomics and protein-protein interaction studies to understand the mechanisms and to obtain new targets for therapy. We will validate gene function by RNAi and using drugs that could be useful to translate our results towards applications.

Expected results

We will identify the molecular mechanisms that determine ferroptosis sensitivity of aggressive melanomas, and use this to design new preclinical therapeutic approaches.

Impact on cancer

The identification of this RAC1-related liability will provide a new framework to understand melanoma metastasis, and to devise new approaches to re-sensitize cancer cells to ferroptosis en route to invasion, or while escaping through the lymph nodes, with the goal of eradicating disseminating cells.

Donatella Romaniello

Alma Mater Studiorum Università di Bologna, Bologna

Neutralizing HER3/NRG1 Axis By A Combination Of Therapeutic Aptamers And Monoclonal Antibodies In HNSCC Organoids

Background

EGFR has been found overexpressed in 80-90% of Head and Neck Squamous Cell carcinomas (HNSCCs) and is associated with poor overall survival and progression-free survival. Surgery and chemo/radiotherapy combined (for HPV-negative HNSCCs) with the anti-EGFR monoclonal antibody cetuximab represent a therapeutic strategy, however, tumors commonly relapse and mechanisms regarding inherent or acquired resistance need to be further elucidated. Immune checkpoint inhibitors like anti-PD1 pembrolizumab and nivolumab have been lately approved by the FDA for recurrent or metastatic HNSCC, but still the role of tumor immunity is not fully understood and currently patients are left with few treatment options.

Hypothesis

Increased HER3 protein level after treatment with cetuximab was observed in HNSCC patients, thus suggesting that this receptor may represent a bypass pathway to the standard of care. Considering that lack of representative models, our hypothesis is to convey our research study into a new viable system to target HER3/NRG1 axis represented by organoids, which can be expanded for long-term remaining genetically and phenotypically stable.

Aims

The aim of this project is to establish a fast and easy way to grow organoids and create a small Biobank of genetically characterized tumors in order to identify new biomarkers, explore mechanisms of drug resistance and apply screening of new drugs for personalized treatment. Particularly, Our goal is to employ PDO to examinate and targeting HER3 axis as a compensatory/bypass signaling pathway responsible for HNSCC resistance to CTX using multiple approaches. Directly neutralizing ERBB3 using an anti-HER3 antibody recently developed by our collaborators. Next, we will explore the use of an innovative nucleic acid-based therapy referred to as aptamers, which we developed upon screening in the last year, and finally with the use of a specific monoclonal antibody targeting the HER3 ligand, NRG1.

Experimental design

In collaboration with Sant'Orsola-Malpighi Hospital we will collect several fresh samples directly from surgery in order to establish and expand few organoids models. All of them will be characterized and HER3, its ligand (NRG1) as well as the other RTKs (receptor tyrosine kinases) expression profile will be defined through several analysis and tested with anti-NRG1 or anti HER3 drugs, including the innovative aptamer, in combination with already approved drugs.

Expected results

Considering the high heterogeneity of HNSCC tumors, the generation of 3D HNSCC organoids will give us an opportunity to have a deeper knowledge of the ERBB signalling and the compensatory signaling pathways. Particularly, organoids will elucidate the involvement of HER3 receptor and its ligand in the onset of cancer

resistance.

Impact on cancer

HNSCC organoids would bypass limitations of the previous old systems and would better resemble morphological and functional HNSCC characteristics. Thus, given the lack of long-term effective treatments, we expect to give a new therapeutic approach and a new hope for HNSCC patients with no treatment option.

Stefano Santaguida

Istituto Europeo di Oncologia I.R.C.C.S. S.r.l., Milano

Mechanistic insights into the consequences of genome instability and aneuploidy on cell physiology

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Background

Aneuploidy, defined as a chromosome number that is not a multiple of the haploid complement, is highly detrimental at both the organismal and cellular levels. Virtually all aspects of cell physiology are impacted by changes in the cell's proteome brought about by an abnormal karyotype. Importantly, aneuploidy is associated with cancer. The aneuploid state is an almost universal feature of cancer cells and its high frequency in tumors is a hallmark of the malignant state. The goal of my work is to elucidate the impact of aneuploidy on cell physiology by identifying and characterizing at the molecular level the pathways deregulated in aneuploid cells.

Hypothesis

To shed light on how aneuploidy contributes to tumorigenesis, it is crucial to determine how chromosome imbalances directly impact normal cells and to determine the immediate consequences of aneuploid karyotypes on cellular functions. Gaining this knowledge will be critical for determining how aneuploidy affects cell physiology in untransformed cells, as well as to start to deconstruct how oncogenic transformation is surpassing aneuploidy-induced cellular stresses. Therefore, we will be providing a detailed molecular picture of the immediate consequences of chromosome imbalances in untransformed cells. Further, we will elucidate how oncogenic transformation is surpassing cellular stresses associated with aneuploidy.

Aims

Over the course of the next 5 years, we will:

1. Perform a mechanistic analysis of proteotoxic stress in aneuploid cells;
2. Deconstruct the molecular basis for aneuploid cell clearance by immune cells;
3. Identify and characterize synthetic lethal interactions of the aneuploid state.

Experimental design

A major goal is to understand, at the molecular level, the events taking place in untransformed aneuploid cells as a consequence of proteotoxic stress and to gain insights into why and how cancer cells are protected from stresses resulting from imbalances in protein composition (Aim 1). Further, we will define the molecular mechanisms underlying immune-mediated aneuploid cell elimination focusing on why changes in chromosome number elicit immune recognition and how malignant transformation is able to bypass immune surveillance of aneuploid cells (Aim 2). Finally, by employing an unpublished library of untransformed aneuploid cell lines that I recently generated, we will identify genes that are synthetic lethal with the aneuploid state (Aim 3).

Expected results

Our novel approach will provide:

- Mechanistic insights into the consequences of proteotoxic stress in aneuploid cells;
- Unique details of the pathways conferring resistance to proteomic changes in cancer cells;
- A molecular analysis of the mechanisms by which aneuploid cells are cleared by the immune system;
- Critical insights on how malignant transformation bypasses immune surveillance of aneuploidy;
- Unprecedented characterization of novel genetic alterations synthetically lethal with models of aneuploidy.

Impact on cancer

More than 90% of all solid human tumors are aneuploid. Understanding the consequences of aneuploidy on cell physiology could provide a novel target in cancer therapy. Further, deciphering how malignant transformation bypasses aneuploidy-associated stresses could shed light on cellular pathways required for the proliferation of aneuploid cancers and could illuminate the path for the developments of therapeutic interventions targeting the aneuploid state of cancer.

Jolanda Sarno

Fondazione M. Tettamanti M. De Marchi Onlus, Monza

Developmental heterogeneity as a key determinant of treatment resistant cells in childhood acute lymphoblastic leukemia

Background

Despite the exciting milestones achieved in the last decades for the treatment of acute lymphoblastic leukemia (ALL), relapsed ALL still remains one of the leading causes of cancer-related death in children. To further improve childhood ALL outcomes, it is critical to identify and understand cellular populations causing treatment failure. Minimal residual disease (MRD) detection after remission induction therapy negatively affects outcome and its measurement is used as predictor of relapse in risk-assignment. MRD cells are rare thus requiring a single cell approach to find and query them. Current clinical MRD approaches do not inform why these leukemia cells are resistant. I herein propose a longitudinal MRD sample analysis at single-cell resolution of a large cohort of patients to identify which are the resistant cells and which is the best way to effectively target them.

Hypothesis

Based on previous findings and preliminary data, I hypothesize that developmental heterogeneity is crucial in treatment failure. Diagnostic pre-B ALL cells relying on active pre-BCR signaling are treatment resistant and represent MRD cells. These cells might persist during treatment due to their metabolic flexibility. Moreover, other sub-clones might also accumulate genetic alterations that lead them to emerge under the pressure of the treatment and be therefore responsible of the relapse.

Aims

To prove my hypotheses, I aim to first identify relapse associated cells at early MRD timepoints and characterize their features. Second, I will investigate mechanisms of resistance to glucocorticoids, drugs heavily used during the remission induction therapy, by focusing on metabolic rewiring of resistant cells. Finally, I aim to determine the clonal identity and gene signature of relapse-associated cells.

Experimental design

I have collected an unprecedented cohort of 50 B-ALL patient samples, at different timepoints of treatment: diagnosis, MRD (day 8 and day 15) and relapse. These samples will be profiled using multi-omics approach. By single cell proteomic analysis, I will identify relapse associated cells to determine if they are already present at the time of diagnosis and persist due to treatment resistance or if they emerge during therapy. On a subset of these samples (n=10), I will study treatment induced metabolic flexibility at single cell level to uncover metabolic heterogeneity. Finally, I will integrate all this information with mutational identity and transcriptome analysis of resistant cells.

Expected results

The comprehensive understanding of phenotype, signaling, cellular metabolism, mutations, and gene signature of relapse-associated cells in clinically annotated longitudinal samples, will unravel novel and key insights on how resistant cells are selected and/or adapted during therapy.

Impact on cancer

On a broader level, the integration of a qualitative evaluation of MRD with the quantitative detection performed with standard methods will further improve the risk stratification of the patients and inform about mechanisms associated with treatment failure. Identifying which cells to target at early stages of resistance will unlock actionable therapeutic options, ultimately preventing relapse and improving the outcome of children with B-ALL.

Martin Schaefer

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Interactions between tumor and microenvironment: contributions to molecular alterations and cancer subtype localization

Background

Colon cancer comprises a heterogeneous group of diseases with several defined subtypes that vary in their molecular characteristics, clinical outcome, and positional preference towards the left or right colon. Cancer is an evolutionary process and the molecular alteration profile reflects the adaption of the tumor to its microenvironment. Tools from population genetics help to understand, which of the typically large number of molecular alterations contributes to tumor initiation and progression.

Hypothesis

Metabolite and microbe concentrations vary along the colon. These changes in the microenvironment could have an impact on carcinogenesis and therefore contribute to the positional biases of tumor subtypes within the colon.

Aims

We will use different computational approaches to identify traces of microenvironmental exposure in cancer genomes, epigenomes and transcriptomes. We will thereby identify components of the microenvironment contributing to carcinogenesis and to the positional biases of tumor subtypes within the colon.

Experimental design

Using tools from population genetics, we will first implement the computational methods to detect genes or genomic regions that are significantly alterated (or depleted in alteration) in different colon positions. We will then implement methods to extract viral, bacterial or fungal reads from tumor genomes or transcriptomes. We will apply these methods to publicly available colon cancer data from the Cancer Genome Atlas (TCGA) and other resources to test if genes that mediate the interaction with the environment (e.g. metabolic genes) undergo alterations in a position-specific manner or if the exposure to viruses, bacteria, or fungi changes along the colon. We will then experimentally verify that the microenvironmental exposure is indeed causing the differences in the alteration profiles along the colon.

Expected results

We will investigate which changes in the microenvironment along the colon (such as the composition of microbes or metabolites) contribute to the differences in the molecular characteristics between left and right colon. We expect to obtain a list of metabolites as well as bacteria, viruses, and fungi that impact carcinogenesis preferentially leading to one subtype of cancer or the other. We will experimentally validate these findings. Finally, this work will lead to the development of a set of computational tools addressing important problems in cancer genomics.

Impact on cancer

The newly developed computational tools will solve important problems in the cancer genomics community and will help to uncover novel causative alterations contributing to cancer initiation and progression. By

understanding how environmental factors spatially bias colon cancer subtype formation, we will potentially uncover novel carcinogens and actionable links between the tumor and its microenvironment. This could lead to novel strategies of cancer prevention and therapy.

Alberto Servetto

Università degli Studi di Napoli "Federico II", Napoli

Exploring chromatin remodeling and transcriptional profiles causing resistance to CDK4/6 inhibitors in ER+ breast cancer

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Background

Combination of CDK4/6 inhibitors and endocrine therapy represents the gold standard treatment for patients with advanced ER+/HER2- breast cancer (BC). Despite a robust clinical benefit, mechanisms of resistance eventually occur and all patients experience disease progression. Several genomic alterations have been associated with resistance to CDK4/6 inhibitors. However, prior studies using targeted or whole exome sequecing (WES) methodologies did not fully recapitulate the landscape of such resistance. Therefore, a profound understanding of mechanisms underpinning resistance to CDK4/6 inhibitors is an unmet clinical need.

Hypothesis

We hypothesize that investigating single-nucleotide variants (SNVs) of non-coding regions of the genome, non-mutational epigenetic reprogramming, chromatin remodelling and consequent changes in gene expression profiles, non-detectable by previously used DNA-sequencing approaches, may clarify how breast cancer cells adapt to CDK4/6 inhibitors.

Aims

Aim 1: To dissect 3-D chromatin landscape and SNVs responsible for palbociclib resistance. Aim 2: To investigate transcriptional activity of YY1, the role of FOXA1 and ERα signalling in palbociclib-resistant cells. Aim 3: To model clinical resistance to CDK4/6 inhibitors using ex vivo tumor organoids and patient-derived xenografts (PDXs).

Experimental design

We generated ER+/HER2- palbociclib-resistant T47D and MCF7 BC cells (T47D-PR and MCF7-PR) using increasing doses of the drug until resistance was achieved. Aim 1: High-throughput Chromosome Conformation Capture (Hi-C) and Whole Genome Sequencing (WGS) will be used to detect, respectively, the differential 3-D chromatin organization and SNVs in palbociclib-sensitive and -resistant cells. Aim 2: Since our preliminary data suggest a role for YY1 and FOXA1 in transcriptional changes associated with palbociclib resistance, ultimately leading to loss of dependency on ERα signaling, we will perform ChIP-Seq, RNA-Seq, mass spectrometry (MS) and loss-of-functions studies in T47D-PR, MCF7-PR and parental cells. Aim 3: We will establish patient-derived organoids (PDOs) and xenografts (PDXs) from metastatic breast cancer biopsies collected from patients progressing on CDK4/6 inhibitors. PDOs will be subjected to WGS, RNA-Seq and drug screening studies.

Expected results

Aim1: Integration of WGS and Hi-C results will elucidate to what extent SNVs uniquely present in palbociclib-resistant cells, by altering the binding of transcription factors (TFs) to DNA, affect 3-D chromatin architecture and gene expression. Also, we will identify the TFs critically involved in resistance to CDK4/6 inhibitors. Aim

2: ChIP-Seq studies will reveal the differential genomic distribution of YY1, FOXA1 and ERα in palbociclib-sensitive and -resistant cells. Next, our studies will clarify whether YY1 knockdown restores palbociclib sensitivity in vivo and abrogates enhancer reprograming and transcriptional profiles associated with palbociclib resistance. MS analysis will detect the differential ERα/FOXA1 nuclear interactome in palbociclib-resistant and -sensitive cells. Aim 3: WGS and RNA-Seq of PDOs will identify SNVs and transcriptional reprogramming occurring in patients progressing on CDK4/6 inhibitors. Drug screening will uncover therapeutic vulnerabilities of CDK4/6 inhibitors-resistant ER+ BC. In vivo studies with PDXs will reveal the effect of novel treatments in overcoming CDK4/6-inhibitors resistance.

Impact on cancer

Our study aims to dissect the genomic alterations, epigenetic processes and TFs causing failure of treatment with CDK4/6 inhibitors. Results from our research would provide a rationale for the development of clinical trials investigating innovative therapies for patients with metastatic ER+ BC.

Giorgia Simonetti

Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) S.r.l. I.R.C.C.S., Meldola

Exploring polyamine metabolism as selective vulnerability for therapeutic combinations in acute myeloid leukemia

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Background

Acute myeloid leukemia (AML) cells undergo metabolic reprogramming and are susceptible to metabolic changes, as induced by Venetoclax (Ven) and Azacitidine (Aza) combination, the current standard of care for elderly/unfit patients that, however, does not cure them. Among AML metabolic hallmarks, we recently uncovered alterations in polyamine metabolism that also shapes tumor-mediated immune response, while being conditioned by the microbiota.

Hypothesis

Recent findings and our preliminary data suggest potential selective vulnerabilities to inhibition of polyamine metabolism across AML molecular subtypes and in combination with Ven/Aza. The definition specific leukemia metabolic dependencies may definitively overcome on-target off-tumor toxicities that currently hamper the clinical applicability of therapeutics in the field of cancer cell metabolism and open a therapeutic window for combined Ven/Aza/polyamine inhibition in AML. Moreover, we hypothesize that a reinforced anti-leukemia T cell response and the microbiota composition may contribute to treatment efficacy when inhibiting polyamine metabolism (also in combination with Ven/Aza). Finally, we believe that preclinical models accounting for the complexity of reactions occurring through tumor-host interactions may relieve the repetitive inconsistency between preclinical and clinical results on metabolism-targeting agents.

Aims

The project aims to explore polyamine metabolism as selective vulnerability for therapeutic combinations in AML. Specifically, it aims to: (i) generate 3D bone marrow models suitable to address the metabolic perturbations occurring in the leukemia microenvironment and reliably evaluate the efficacy of therapeutic combinations; (ii) study the pathogenic role of dysregulated polyamine metabolism in AML, both as leukemic fuel and immunosuppressive mechanism; (iii) exploit polyamine targeting to improve Ven/Aza combination efficacy, by unraveling specific dependencies, towards metabolic-oriented personalized AML therapies.

Experimental design

To accomplish the research aims, the project is developed along three integrated work-packages (WPs). In WP1 we will structure and characterize autologous multicellular 3D bone marrow models obtained by combining cell bioprinting and cultures. In WP2 we will inhibit polyamine metabolism by pharmacological approaches and cell engineering and study its functional consequences on leukemic cells and the immune response both ex vivo, in the 3D model and in vivo, in immunocompetent mice. In WP3, we will study the combination of Ven/Aza and polyamine metabolism inhibition ex vivo and in vivo, and we will predict biomarkers of response to the triplet, taking into account the disease molecular feature and the microbiota contribution to the polyamine reservoir.

Expected results

The project will uncover the functional role of polyamine metabolism in AML. By evaluating drugs already known to be suitable for clinical combinations, capturing selective vulnerabilities of AML molecular subgroups and defining predictive markers of ex vivo response, the results will enable a direct translation of our metabolic-oriented personalized approach into clinical trials of Ven/Aza/polyamine metabolism inhibitors. Moreover, we will provide a preclinical model ameliorating personalized drug testing and accelerating drug development or repurposing.

Impact on cancer

The project answers the urgent medical need of early and personalized interventions able to induce deep and durable clinical responses, while sparing toxicities in elderly/unfit AML patients that currently have a 5-year survival of 5-10% and no therapeutic options at disease relapse/refractoriness.

Federica Sodano

Università degli Studi di Napoli "Federico II", Napoli

targeting arginaSe 2 tO disrUpT tHe imMunosUppreSsive Tumour microEnvironMEnt and pRomote Gastric cancEr response

Background

Gastric cancer (GC) is the fifth most common malignancy and the third leading cause of cancer death worldwide. Immunotherapy based on immune checkpoint inhibitors, such as PD-1 and PD-L1, has undoubtedly revolutionized the treatment of some previously incurable cancers and has become one of the mainstays of innovation in cancer therapy. However, only a small percentage of patients can benefit from immunotherapy because of the establishment of resistance mechanisms as those related to the immunosuppressive tumor microenvironment (TME). It has been recently reported that melanoma-associated fibroblasts impair CD8+ T cell function and modify expression of immune checkpoint regulators via increased arginase (ARG) activity. Moreover, ARG isoform 2 (ARG2) controls regulatory T (Treg) cell metabolic fitness and correlates with their immunosuppressive function in cancer.

Hypothesis

ARG2 is therefore a valuable target for T-cell-based cancer immunotherapies. Because ARG1 and ARG2 are structurally similar, the development of an ARG2-specific inhibitor has not borne fruit. Taking advantage of the different subcellular localization of the two isoforms, specific inhibition of ARG2 could be developed by synthesizing molecular hybrids capable of delivering the most promising ARG inhibitors (ARGi) into mitochondria.

Aims

This project proposal aims to both identify new and more potent inhibitors (nARGi) and molecular hybrids targeting ARG2 to improve the success rate of immunotherapies and contribute to the development of patient-centred personalized therapy.

Experimental design

The design of new and more potent inhibitors (nARGi) will be carried out using computer-assisted drug design techniques. The identified scaffolds (nARGi) will subsequently be targeted to mitochondria (mt-nARGi) by covalent bonding with high trophism vectors for mitochondria. As an innovative strategy, the effect of ARG inhibitors on the native TME and GC-specific immune response in GC-Patient Derived Organoids will be evaluated.

Expected results

The expected results from evaluating the effect of ARG inhibitors on TME composition and activation and GC-specific immune response will help elucidate the onset, development, and metastasis of GC. In addition, for the first time, organoids derived from GC patients (GC-PDOs) will be used as a model.

Impact on cancer

The identification of new molecular hybrids targeting ARG2 would aid disease management, improve the success rate of immunotherapies, and contribute to the development of target therapies, with benefits in

quality and life expectancy for patients with GC and beyond.

Giovanni Sorrentino

International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste

Role of tissue mechanics in the progression from NASH to liver cancer

Background

NASH is a condition characterized by inflammation of the liver with concurrent hepatic fat accumulation. In about 25% of patients, NASH progresses into cirrhosis, a degenerative and irreversible disorder that results from excessive scar tissue accumulation and strongly associates with development of liver cancers. As a direct effect of the excessive collagen remodelling and crosslinking, the mechanical environment in NASH liver progresses inexorably toward a profoundly altered state characterized by increased tissue stiffness.

Hypothesis

While the interplay between oncogene-expressing cells and their surrounding microenvironment is pivotal to initiate tumorigenesis, whether genetic lesions conspire with the NASH-associated stiff ECM to promote cancer development remains a medical enigma.

Aims

My proposed research aims at filling this gap of knowledge and is directed at scrupulously dissecting the highly neglected, yet crucial, biomechanical events occurring during the transition from NASH to liver cancer, with the ultimate goal of understanding the mechanisms and the functional contribution of aberrant tissue stiffness on HCC development.

Experimental design

A combination of multiple cutting-edge experimental tools will be used. The access to unique clinical material will allow the creation of a living biobank of mouse- and patient-derived liver organoids to be used for the design of disease models able to recapitulate ex vivo the biomechanical features of the original tissues in ways that have previously been impossible. In this respect, an unprecedented technology to model the multi-step nature of the NASH to liver cancer transition, based on genetically-engineered liver organoids cultured in mechano-modulatory hydrogels, will be introduced and will serve as a discovery resource to probe the effect of ECM stiffness on liver cancer development. This multifactorial and experimentally amenable set-up represents a breakthrough in the field of liver cancer, due to the possibility to combine, for the first time, genetic, metabolic and environmental factors in the same culture system. Genetically engineered and dietary-induced mouse models of fatty liver disease, faithfully mirroring the entire human NAFLD spectrum, will be used to investigate the role of tissue mechanics on NASH to liver cancer progression in vivo. Moreover, an integrative and multi-layered approach combining high throughput transcriptomics, metabolomics, epigenetics, bioinformatics, will be applied to mouse and human organoids and liver samples to resolve the molecular mechanisms underlying the biomechanical basis of NASH to liver cancer progression and to identify novel potential therapeutic targets.

Expected results

I expect to unequivocally demonstrate that aberrant increase in liver stiffness actually anticipates overt disease and may play a causal role in liver cancer onset. This will allow me to design innovative therapeutic

approaches to prevent liver cancer development in patients with advanced metabolic liver disorders.

Impact on cancer

Overall, this project is highly ambitious and proposes the innovative concept of targeting liver mechanotransduction to obtain tangible clinical benefit for NASH patients. Owning on its original and technologically advanced nature, this proposal aims to take a step forward in the field of mechanobiology and has the potential to break through the existing barriers that limit the current approaches for the treatment of NASH and the prevention of liver cancer development.

Francesco Spallotta

Istituto Pasteur Italia - Fondazione Cenci Bolognetti, Roma

Metabolic regulation of the DNA demethylation enzymatic machinery in pancreatic cancer

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Background

Epidemiological studies have pointed out a correlation between dysmetabolism and pancreatic cancer. Dysmetabolism and the consequent altered cellular metabolite availability impact on the epigenetic landscape. DNA methylation and its enzymatic machinery are particularly sensitive to metabolic changes. Consequently, the alteration of cytosine methylation pattern affects cell transcriptome and leads to the establishment of the so-called "epi-metabolic memory". My recent works have shed light on the stroma as a sensor of dysmetabolism and as a keeper of epi-metabolic memory. Pancreatic ductal adenocarcinoma (PDAC) is characterized by a scarce proportion of malignant cells and a preponderant desmoplastic stroma, abundant in extracellular matrix deposition, cancer-associated fibroblasts (CAFs), disorganized endothelium and exhausted immune cells. PDAC stroma hampers pancreatic tissue homeostasis and plays a pivotal role in refractoriness to therapy.

Hypothesis

We postulate that chronic dysmetabolism alters the activity of the DNA methylation enzymatic machinery leaving an epi-metabolic inheritance which harnesses pancreatic tumorigenesis. We specifically hypothesize that CAF activation and function in PDAC stroma are modulated by this dysmetabolism-dependent epi-metabolic memory, thereby widely contributing to pancreatic cancer progression and therapy failure.

Aims

The proposal aims at: 1) identifying an epi-metabolic signature associated with impairment of DNA methylation related enzymes caused by dysmetabolism in pancreatic tumorigenesis; ii) understanding the function of DNA methylation related enzymes during PDAC stroma development; iii) revealing the cluster of epi-metabolic genes associated with PDAC reversible/persistent epi-metabolic memory; iv) improving PDAC therapeutic strategies targeting dysmetabolism and DNA methylation-related enzyme to modulate the stromal compartment.

Experimental design

These objectives will be pursued through a focused approach consisting in: 1) exposure of a genetically engineered mouse model of pancreatic tumorigenesis to high-fat diet, which mimics a pre-metabolic syndrome and causes dysmetabolism; 2) in vivo evaluation of PDAC progression upon dysmetabolism by bioluminescence imaging; 3) epi-metabolic characterization of the interaction between pancreatic stroma and tumor; 4) cellular modeling of the interaction between pancreatic CAF and tumor cells; 5) pharmacological specific targeting of epi-metabolic mediators in combination with gemcitabine and evaluation of PDAC progression.

Expected results

This proposal will reveal how dysmetabolism impacts on pancreatic tumorigenesis and progression and will

shed light on novel epi-metabolic combinatorial therapeutic approaches with gemcitabine impacting on stroma organization to halt PDAC.

Impact on cancer

This study will provide novel insights into epi-metabolic mediators which can help to stratify dysmetabolic PDAC patients in a precision medicine perspective. It may also lead to the design clinical trials testing combination of epi-metabolic approaches and standard chemotherapies in the growing PDAC patient population with a history of dysmetabolism.

Elisa Taiana

Fondazione I.R.C.C.S. Ca' Granda Ospedale Maggiore Policlinico, Milano

Investigation of the biological, molecular, and clinical relevance of NONO in multiple myeloma

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Background

Multiple Myeloma (MM) is an haematological cancer characterized by the malignant proliferation of bone marrow plasma cells (PCs). Clinically, resistant clones arise upon the selective pressure of subsequent lines of therapy and, notwithstanding the remarkable improvement in treatment, MM still remains an incurable disease leading to the continuing need to identify novel prognostic biomarkers. In this regard, we demonstrated that the lncRNA NEAT1, the fundamental structural scaffold of paraspeckle (PS) organelles, is crucial for MM cells survival and that its targeting triggers anti-tumor activity in MM. NONO represents an essential protein for NEAT1 stability and PSs assembly. It binds to DNA, RNA, and proteins and it is involved in almost every step of gene regulation. Furthermore, NONO has been found dysregulated in many types of cancer, and, concerning MM, the only available data have recently included NONO in a prognostic signature, very reproducible in different MM databases.

Hypothesis

Besides its essential role for PSs assembly and stability, NONO could carry out important activities independently from PSs. This fact led me to speculate that its targeting could trigger several and independent effects in MM cells and that a better understanding of its roles in MM could make it therapeutically valuable and useful for the development of novel pharmacological approaches.

Aims

The overarching aim of my proposal is to elucidate the biological, molecular and clinical relevance of NONO in MM, also inspecting the impact of NONO targeting on MM therapy.

Experimental design

I will investigate the biological role of NONO in MM by knocking-down (KD) its expression in in vitro experimental models of MM disease, in the presence or absence of bone marrow stromal cells, known to support MM proliferation and confer drug resistance. Furthermore, results obtained from in vitro experiments will be consolidated by means of in vivo studies as well.

I plan to identify NONO's DNA, RNA, and protein interactors that will be of relevance to clarify its oncogenic role in MM. To this aim RNA-Seq, RIP-Seq, ChIP-Seq, ChIRP-Seq, as well as proteomic approaches will be adopted. Relevant identified targets will be further functionally and molecularly investigated to evaluate their contribution to the disease.

The translational significance of NONO targeting in MM will be studied taking advantage of a drug-based synthetic lethality screening of NONO-KD cells. Promising combinations will be validated also in in vivo models.

Expected results

I expect to shed light on NONO mechanism of action in MM, by dissecting its involvement at genomic,

transcriptomic and protein levels. Moreover, I foresee to identify promising synthetic lethality combinations in vitro and in vivo, therefore contributing to the development of novel approaches for MM treatment.

Impact on cancer

My proposal will contribute to clarify the pathological role of NONO in MM and will represent an important step forward in the hemato-oncology field, contributing to identify putative druggable targets of this fatal malignancy. Moreover, considering that NONO is deregulated and plays an oncogenic role in several human cancer, obtained results will have implications also for other research areas involving hematologic malignances and solid tumors.

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Exploring how endothelin-1/PIEZO axis intersects mechanical forces to fuel PARP inhibitor resistance in ovarian cancer

Background

In the majority of high-grade serous ovarian cancer (HG-SOC) patients PARP inhibitors (PARPi) resistance is an urgent clinical issue. Biomechanical and biochemical inputs, as those actioned by the endothelin-1 (ET-1) receptors (ET-1R), belonging to the G-protein coupled receptor (GPCR) family, and intercepted by the mechanotransducer YAP, confer PARPi resistance. In addition, the ion channel PIEZO1 has been linked to YAP-driven mechanoresponsive pathways. Among the mechanosensitive DNA damage checkpoints highly expressed in HG-SOC, ATR and POLθ represent emerging therapeutic targets. Intriguingly, PARPi and POLθ inhibitors (POLθi) induce the cGAS-STING pathway and activate the immune system. Because the fine mechanism by which the mechanical cues fuel PARPi-therapy evasion is not clarified, exploring how the ET-1R/PIEZO1/YAP signaling connects cellular and nuclear mechanics to DNA Damage Response (DDR), offers the rational to develop novel combinational strategies.

Hypothesis

Our preliminary data unveil that the ET-1R/PIEZO1/YAP mechanosignaling, intersecting extracellular mechanics, enhances the expression of DNA damage checkpoints and the release of pro-inflammatory cytokines. Therefore, we aim to explore how the integration between ET-1R/PIEZO1/YAP mechanoaxis and mechanical forces, regulating DNA damage checkpoint activity, as ATR and POLθ, and ensuring the mechanical coupling of cytoskeleton to the nucleus, can generate a persistent PARPi-tolerant state in multidimensional models. ET-1R blockade, interfering with the ET-1R/PIEZO1/YAP-mediated PARPi evasion, could offer a valid therapeutic companion for PARPi and other emerging therapies, as ATR inhibitors (ATRi), or POLθi. In addition, ET-1R antagonists, leveraging the cGAS-STING pathway and upregulating PD-L1, can be combined with immunotherapy (ICI), representing a new avenue to overcome PARPi resistance.

Aims

In patient-derived (PD) HG-SOC preclinical models we will:

- Explore how the integration between the ET-1R/PIEZO1/YAP mechanosignals and mechanical forces, orchestrating DDR players, as ATR and POLθ, impacts on the transcriptional activity involved in PARPi response;
- Examine how ET-1R/PIEZO1/YAP circuit and mechanical inputs, triggering dynamic changes in nuclear structure and morphology, reprograms the tumor microenvironment directing PARPi evasion;
- Evaluate how ET-1R blockade, when combined with PARPi and emerging therapies, as ATRi, or POLθi, or ICI, may overcome PARPi resistance.

Experimental design

PARPi-sensitive and -resistant PD HG-SOC 3D primary cultures, spheroids, and co-culture models will be used to dissect the mechanism by which the ET-1R/PIEZO1/YAP-driven mechanosignaling, at the cytosolic

and nuclear level, confers PARPi resistance. The single-cell transcriptome of HG-SOC and immune cells will be characterized by sc-RNA-seq. Nuclear dynamics will be evaluated by changes in selected protein expression and by nuclear deformation analysis. Pertinent HG-SOC mouse models will be used to examine the therapeutic efficacy of ET-1R blockade in combination with PARPi, mechanotherapeutics, or ICI. Changes in the immune system profile following therapy will be assessed with high spatial and temporal resolution imaging.

Expected results

Unveiling ET-1R-mediated mechanosignaling will contribute to disentangle the root of the onset of PARPi evasion, highlighting the potential applicability of PARPi with ET-1R antagonists with ATRi, or POLθi, or ICI.

Impact on cancer

Deciphering the mechanical nucleo-cytoskeletal force transmission holds the potential to transfer into the clinic ET-1R antagonist/PARPi combination with mechanotherapeutics, and to expand the combination with ICI, as standard of care in tumors poor responsive to ICI, as HG-SOC.

Anna Urciuolo

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Modeling and targeting the mechanisms underlying cancer-cachexia using human neuromuscular system in vitro models

Background

Cachexia is experienced by 80% of patients with cancer. This metabolic wasting syndrome is characterized by anorexia, inflammation and severe loss of skeletal muscle. The loss of skeletal muscle mass and strength is considered the most important clinical event in cancer cachexia, and a key predictor of poor outcomes. Cancer cachexia is responsible of worsening patient quality of life, inducing lower tolerance to anticancer treatments, a drastic reduction in mobility and feeding ability, increased use of healthcare resources, and accounts for up to 30% of cancer deaths. Nutritional supplementation is unable to reverse this syndrome. Effective treatments for cachexia are still lacking. Molecules released within the systemic circulation from the cancer or as consequence of it from other organs are considered muscle cachexia mediators. These mediators can trigger and sustain loss of muscle mass by activating catabolic processes in muscle and inhibiting muscle protein synthesis.

Hypothesis

To date, it is still unclear what initiates the cachexia cascade in cancer patients and which molecules can be targeted to counteract muscle cachexia. To increase our knowledge and the chance to accelerate testing single or combined anti-cachexia therapies for translational studies, the understanding of the signaling pathways involved in human biology of muscle cachexia is crucial. I hypothesized that the use of an advanced functional human skeletal muscle in vitro model in combination with cancer-derived pro-cachectic samples could surpass the limit of studying human biology of muscle cachexia in patients.

Aims

With this project we aim to identify the molecular drivers of skeletal muscle mass and function loss mediated by cancer-cachexia in a human-specific fashion. This is based on the use of functional human skeletal muscle in vitro models which can mimic atrophic and hypertrophic responses in the context of muscle cachexia. By validating targetable molecular pathways, we will develop novel strategies to block cachexia-mediated muscle unbalance and/or restore skeletal muscle mass and function.

Experimental design

This project is based on the treatment of human neuromuscular organoids derived from human induced pluripotent stem cells with muscle cachexia secreted mediators. Since the organoid platform allows the derivation of mature and functional skeletal muscle models, we will investigate cachectic phenotype at functional, cellular, and molecular levels. The study is designed to i) dissect and ii) target muscle cachexia at molecular level firstly in a contest of iii) gold standard administration of cachexia mediators, and then vi) by introducing human circulating factors present in the serum of cancer patients.

Expected results

We expect to reach the following results: 1) Development of an in vitro human neuromuscular system

platform to study human muscle cachexia; 2) Identification of targetable molecules to counteract muscle cachexia phonotypes; 3) Validation of mechanisms and efficacy of our targetable approach using cachectic mediators derived from cancer patients.

Impact on cancer

This project will increase our knowledge on the human biology of muscle cachexia and will identify targetable human cachexia molecular players to make a shorter road for translating these experimental studies successfully in the clinic.

Eleonora Vannini

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Boosting protective immunity with the recombinant protein CTX-CNF1 to treat Glioblastoma

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Background

Glioblastoma Multiforme (GB) is the most aggressive form of glioma, with an average survival of only 15 months. Current treatments are not effective, thus there is an urgent need to develop new and efficient therapeutic approaches. Recent evidence has demonstrated that the bacterial protein CNF1 can reduce tumoral mass and protects peritumoral tissue from neural dysfunction during GB growth. Despite these promising results, CNF1 is not able to cross the blood-brain barrier (BBB) per se, hampering its translation to the clinic. To enable a systemic administration of CNF1, we developed a chimeric protein conjugating CNF1 with Chlorotoxin (CTX), a peptide already employed in GB clinic as a drug vector for its capacity to cross the BBB. Preliminary data show that a systemic CTX-CNF1 administration i) prolongs survival of glioma-bearing mice and restores their normal motor function, ii) specifically targets and counteracts GB cell proliferation, iii) reduces tumor mass, and iv) boosts T cell infiltration in the peritumoral tissue. This last result is of particular importance because GB is known for being an immunologically 'cold' tumor, displaying very low amounts of infiltrating immune cells in the tumor microenvironment.

Hypothesis

We hypothesize that CTX-CNF1 treatment inhibits GB growth in mice not only by directly targeting GB cells in the brain, but also by boosting immune system activation and anti-tumor activity.

Aims

This project aims to investigate whether CTX-CNF1 may represent an innovative therapeutic approach to treat GB and, potentially, other tumors by acting as an immune-stimulatory drug.

Experimental design

We will develop a complete preclinical plan with experiments on cell cultures, mouse models and human brain sections. We will evaluate i) brain architecture and functionality before and after the treatment with CTX-CNF1 (in mouse and human); ii) the safety of CTX-CNF1 administration; iii) which are the molecular mechanisms triggered by the recombinant protein that may stimulate protective immunity; iv) the effect of CTX-CNF1 + monoclonal antibodies against immune checkpoint inhibitors (ICI) vs monotherapy ICI antibody strategies; v) the effect of CTX-CNF1 in human autograft lymphocyte-glioblastoma model.

Expected results

We expect to confirm the high potential of CTX-CNF1 therapy for the treatment of GB. Our study will represent a complete preclinical investigation, dissecting the overall effect of our recombinant protein on tumor cells, immune system and CNS-resident cells in vivo and in vitro. Importantly, experiments will be carried out in mouse models and in human samples, paving the way to a potential translation to the clinic.

Impact on cancer

GB represents a huge problem for public health because of the lack of effective therapies, the elevated

mortality and the short life expectancy. Its development, fast and destructive, results in an extremely serious and invalidating symptomatology that makes GB a significant social and economic burden for National healthcare systems. Acting as an immuno-stimulatory drug, CTX-CNF1 may represent an effective brand-new approach for the treatment of GB that might improve life quality and expectancy of GB patients. The results of our study will clarify the translational potential of CTX-CNF1, and will pave the way for the development of novel therapeutic strategies against CNS tumors.

Rossella Venditti

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Dissecting the role of membrane contact sites in cancer progression and drug resistance

Background

In recent years membrane contact sites (MCSs) have been identified as a crucial route for inter-organellar exchange of ions and lipids and, more generally, for important cellular functions including membrane trafficking and secretion. MCSs are highly dynamic structures where different organelles come into close apposition (10-30 nm) without any fusion events. We found that affecting MCS stability increases the secretion of pro-invasive and pro-metastatic factors while interfering with proteins involved in their maintenance and stability can increase (when negatively regulated) or reduce (when positively regulated) cell motility. We also found that the lipid transfer protein CERT, responsible for the non-vesicular transport of ceramide, negatively regulates MCS formation. It has been shown that CERT inhibition causes increased ceramide levels in the ER and we found that MCS stabilization is directly dependent on ceramide content. Since ceramide is an active sphingolipid precursor frequently altered in breast cancer, we propose identifying ceramide sensors and interactors at the MCS interface that can be relevant novel therapeutic targets for intervention.

Hypothesis

Although the study of MCSs (in particular ER-Golgi and ER-endosome contacts) has clearly demonstrated their role in orchestrating diverse cellular functions and the catalog of MCS components is steadily increasing, a systematic analysis of their composition, regulation and function will be pivotal in understanding cancer cell biology, cancer development and progression.

Aims

The aim of this proposal is to explore and to provide insights into how ER-Golgi and ER-endosome MCSs are regulated during cancer development and how the functions regulated by these MCSs may affect cancer progression and dissemination mechanisms. In addition to lipid control by MCSs, we will also systematically study MCSs in breast cancer cells and in cell cycle progression prompted by the observation that these ER-mediated contacts are sensitive to control by kinases involved in metastatic spreading and chemo-resistance. Finally, we will investigate how maintenance and regulation of contact sites affect cell motility and invasion as well as asymmetric cell division of breast cancer stem cells. The aim of this proposal is to explore and to provide insights into how ER-Golgi and ER-endosome MCSs are regulated during cancer development and how the functions regulated by these MCSs may affect cancer progression and dissemination mechanisms.

Experimental design

To address these important open questions, we aim to identify:

1. Regulation of ER-Golgi and ER-late endosome MCSs
2. Composition of ER-Golgi and ER-late endosome MCSs

3. Role of MCSs in cell motility and invasion
4. Role of MCSs in controlling cell division and organelle inheritance.

Expected results

The expected results obtained through this project will significantly impact on understanding how lipid membranes and cancer progression and dissemination are integrated which will represent an important breakthrough in the cancer cell field

Impact on cancer

We believe that a deeper understanding of MCS composition, regulation and function in breast cancer and, in general, in cancer cells will have a major impact on the field of cancer biology both in terms of novel molecular mechanisms to explore as alternative therapeutic pathways and in terms of intracellular organelle communication.

Claudio Vernieri

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Exploiting inducible metabolic vulnerabilities to improve chemotherapy efficacy in triple negative breast cancer

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Background

Triple-negative breast cancer (TNBC) is the most aggressive and deadly subtype of breast cancer (BC). Dysregulation of glucose metabolism and growth factor-mediated signalling has been shown to sustain TNBC bioenergetics and anabolic functions. Consistently, combining cycles of fasting or fasting mimicking diets (FMDs) with cytotoxic chemotherapy produced synergistic anticancer effects in TNBC murine models. We recently showed that the FMD is well tolerated by cancer patients, and induces a significant reduction of blood glucose and growth factor concentration, combined with a previously undetected increase of plasma branched chain amino acids (BCAAs). Preliminary in vitro data indicate that modulating the ratio between extracellular BCAAs can be toxic to several tumor models including TNBC.

Hypothesis

We hypothesize that inhibiting metabolic pathways that are activated by TNBC cells in response to nutrient starvation/BCAA modulation can enhance the anticancer activity of starvation. Identifying those pathways will allow the design of synthetic lethal metabolic approaches to target TNBC metabolic heterogeneity.

Aims

To test our hypothesis, we will produce experimental growth media, collectively referred to as fasting-mimicking conditions (FMCs), which recapitulate FMD-induced metabolic changes, namely reduced glucose/growth factor and increased BCAA concentration. We aim to: 1) identify biological mechanisms responsible for the in vitro anti-TNBC activity of FMCs, alone or in combination with cytotoxic agents; 2) study FMC-induced modulation of crucial metabolic pathways, and the impact of downregulating these pathways on the anticancer effects of starvation; 3) unveil mechanisms through which an imbalanced concentration of extracellular BCAAs is toxic to TNBC cells.

Experimental design

Different human TNBC cell lines will be grown in standard media, and then switched to FMCs, alone or in combination with doxorubicin or cisplatin. We will study the impact of these treatments on cell survival, PI3K/AKT/mTORC1 axis activation and stress response pathways. To identify pathways of resistance to FMCs, we will perform global gene expression and proteomic analyses. Selected metabolic transporters/enzymes that are upregulated during FMCs will be downregulated in in vitro and in vivo (mouse xenografts) TNBC models, and cancer cell survival/tumor growth will be assessed. Crucial results of preclinical experiments will be validated in tumor specimens from TNBC patients undergoing the FMD, plus/minus metformin, in combination with standard chemotherapy in the context of a prospective clinical trial. Finally, we will characterize the molecular mechanisms mediating the cytotoxicity of BCAA imbalance in TNBC cells.

Expected results

Through a combination of population and time-lapse microscopy experiments, as well as of confirmatory in

vivo experiments and analyses in tumor specimens from patients, we will identify new synthetic lethal anti-TNBC metabolic interventions to enhance the anticancer activity of starvation. We will also elucidate the molecular mechanisms responsible for the cytotoxic effects of imbalanced extracellular BCAA concentration, and we will provide first time validation of this approach in animal experiments.

Impact on cancer

This project will elucidate metabolic pathways that TNBC cells activate to survive nutrient starvation, actually a highly promising, experimental anticancer approach. By identifying synthetic lethal metabolic interventions able to target intratumor and intertumor TNBC metabolic heterogeneity, this study will reveal new metabolic anticancer interventions to be tested in future clinical trials.

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Mondrian: multi-omics integrative modelling for stereotactic body radiotherapy in early-stage non-small cell lung cancer

Background

While diagnostic anticipation has contributed to reduce mortality related to non-small cell lung cancer (NSCLC), it still ranks as first among big killers in Oncology. Treatment strategies for Early-stage (ES) disease are surgery or stereotactic body radiation therapy (SBRT), with successful local control rates for both approaches. However, regional and distant failure remain critical in SBRT, and it is paramount to identify predictive factors of response in this clinical setting to identify high-risk patients who may benefit from more aggressive approaches.

Hypothesis

Recently, a better understanding of NSCLC immunobiology has led to the successful use of immune checkpoint inhibitors in locally-advanced and metastatic disease. Therefore, there is increasing interest in translating this approach to ES disease. To date, ongoing investigations have enrolled surgical candidates, and a focus on SBRT is lacking. However, given the well-recognized immunomodulatory ability of SBRT, its combination with immune-checkpoint inhibitors is promising, and could substantially improve the outcomes of poor SBRT responders.

Aims

Given available preliminary evidence from the individual fields of radiomics, genomics and proteomics, the primary endpoint of MONDRIAN is to identify multi-omic biomarkers of SBRT response through advanced computational integration of the above-mentioned information layers. Secondary endpoints include the assessment of novel omic-derived prognostic factors, the design and validation of methodological radiomic and dosomic studies, and the longitudinal validation of gene expression and proteomics profiling between tissue- and liquid biopsy- derived samples.

Experimental design

MONDRIAN is designed as a prospective observational explorative cohort clinical study, with data-driven, bottom-up approach. It is expected to enroll 100 ES-NSCLC SBRT candidates treated at an Italian tertiary cancer center with well-recognized expertise in high-precision RT. To identify predictors specific to SBRT, MONDRIAN will include, and collect data from, patients treated with surgery in a 1:2 ratio, thus achieving a number of 200 operated subjects with comparable clinical characteristics. The project will have an overall duration of 60 months, and will be structured into five main tasks namely, i. Clinical Study, ii. Imaging/ Radiomic Study, iii. Gene Expression Study, iv. Proteomic Study, v. Integrative Model Building.

Expected results

Thanks to its multi-disciplinary nature, MONDRIAN is expected to provide an unprecedented opportunity to characterize ES-NSCLC from a multi-omic and comprehensive perspective, and to elucidate which parameters interact to determine radiosensitive and radioresistant phenotypes.

Impact on cancer

Other than contributing to a mechanistic understanding of the disease, the study will assist the identification of high-risk patients in a largely unexplored clinical setting. Ultimately, this would orient further clinical research efforts on the combination of SBRT and (neo)adjuvant systemic treatments, such as immunotherapy, with the perspective of improving oncological outcomes in this subset of patients.