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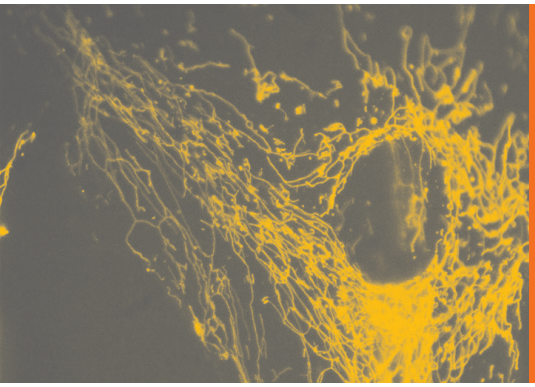
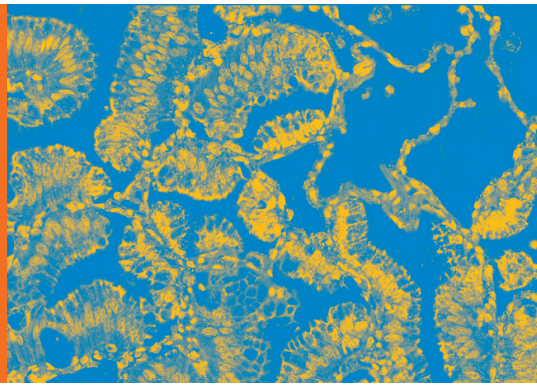
VIRTUAL MEETING
May 27th-29th, 2021

4TH EDITION

METABOLISM & CANCER





ABSTRACTS' BOOKLET

DAY 1: THURSDAY, 27TH MAY

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Session I
REDOX & ENERGY METABOLISM

Chairs: Rodrigue Rossignol (MRGM, Bordeaux, France) **& Sophie Vasseur** (CRCM, Marseille, France)

Keynote lecture
Mitochondrial REDOX signaling and drug modulation

Mike Murphy
MRC Mitochondrial Biology Unit, University of Cambridge, Hills Road, Cambridge, United Kingdom

Mitochondrial redox metabolism is central to the life and death of the cell. For example, mitochondrial production of free radicals and subsequent oxidative damage has long been known to contribute to damage in conditions such as ischaemia-reperfusion (IR) injury in stroke and heart attack. More recently mitochondrial redox changes have also been implicated in redox signalling. Over the past years we have developed a series of mitochondria-targeted compounds designed to ameliorate or determine how these changes occur. I will outline some of this work, which suggested that ROS production in IR injury during stroke was mainly coming from complex I. This led us to investigate the mechanism of the ROS production and using a metabolomic approach we found that the ROS production in IR injury came from the accumulation of succinate during ischaemia that then drove mitochondrial ROS production by reverse electron transport at complex I during reperfusion. This surprising mechanism led up to develop further new therapeutic approaches to impact on the damage that mitochondrial ROS do in pathology and also to explore how mitochondrial ROS can act as redox signals. I will discuss how these unexpected mechanisms may lead to redox and metabolic signals from mitochondria in a range of conditions under both healthy and pathological conditions.

Lecture
xCT-based ferroptosis inducers in the treatment of pancreatic ductal adenocarcinoma – opportunities and challenges

Milica Vucetic, Boutaina Daher, Willian Meira, Scott K. Parks, Yann Cormerais, Célia Gotorbe, Jérome Durivault, Jacques Pouyssegur
Department of Medical Biology, Centre Scientifique de Monaco (CSM), Monaco

Contextualisation of the new type of regulated cell death called "ferroptosis" that has been seen as a new strategy for (re)induction of the cell death program in cancer cells, sparked great optimism in the cancer research community. Cumulative fundamental research over the past century, crowned by the extraordinary work of Stockwell's laboratory and backed up by the intensive research during the last decade, finally got its candidature to be applied in the clinical settings. However, still the special attention has to be placed on the multiple potential mechanisms that confer resistance to this type of cell death.
Preclinical research of our and other laboratories unequivocally showed that the cysteine-starvation is a very potent and highly promising mechanism for ferroptosis induction in pancreatic ductal adenocarcinoma (PDAC) cells. Cysteine involvement in ferroptosis prevention has been seen primarily through its role in the synthesis of glutathione (GSH), the major non-enzymatic antioxidant within the cells. Accordingly, GSH serves as a reducing power for GSH peroxidase 4 (GPx4) which catalyses removal of iron-induced lipid

hydroperoxides and thus prevents ferroptotic cell death. However, increasing number of data point toward GSH-independent role of cysteine in ferroptotic process. Thus, from many different approaches to induce ferroptosis in cancer cells, the ones targeting transporter of oxidized form of cysteine (aka xCT) seems to be the most promising. Data from our previous study have shown that genetic invalidation of the xCT in two different PDAC cell lines leads to complete collapse of GSH, but also cysteine levels in the cells leading to disrupted protein synthesis, unbalanced redox homeostasis, and finally cell death. As expected, this type of cell death was completely prevented by classic ferroptosis inhibitors: lipophilic antioxidants (Vitamin E), alternative donors of cysteine (N-acetylcysteine, GSH) or iron chelators (deferoxamine).

The most surprising data from the study, however, showed complete resistance of the xCT-KO cells *in vivo*. Namely, although highly sensitive to ferroptosis in *in vitro* conditions, xCT-KO cells were able to survive and even to give raise to tumours when implanted in the mice. Potential explanation for this lies in the fact that xCT plays fundamental role in cyst(e)ine import in the conditions where exclusively or almost exclusively oxidized form of this amino acid is present (*in vitro* conditions). *In vivo*, however, although oxidized cysteine is still dominant form, its reduced form can come from many different sources, such as circulation or extracellular space. Furthermore, our data clearly showed that reduced cysteine is provided by neighbouring xCT-proficient cells. According to the data, oxidized-reduced cyst(e)ine shuttle between cancer and neighbouring cells is able to completely prevent ferroptosis of the xCT-KO PDAC cells even *in vitro*.

In conclusion, xCT as a major exchanger glutamate/cystine is fundamental for normal metabolic and redox homeostasis of the PDAC cells. Its disruption/inhibition inevitably leads to ferroptotic cell death. However, the data also suggest that xCT inhibition has to be achieved systematically in order to avoid cell-to-cell interplay.

Keywords: ferroptosis, xCT, cell-to-cell interplay, cyst(e)ine, PDAC.

References:
- Dixon, S. J. *et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell 149,* 1060–1072 (2012).
- Daher, B. *et al. Genetic ablation of the cystine transporter xCT in PDAC cells inhibits mTORC1, growth, survival, and tumor formation* via *nutrient and oxidative Stresses. Cancer Res.* 79, 3877–3890 (2019).
- Meira *et al. A cystine-cysteine intercellular shuttle prevents ferroptosis in xCTKO pancreatic ductal adenocarcinoma cells. cancers* 13(6): 1434 (2021).
- Vucetic, M. *et al. Together we stand, apart we fall: how cell-to-cell contact/interplay provides resistance to ferroptosis. Cell Death & Disease* 11, 789 (2020).

Selected talk

Exploiting a redox bottleneck to combat drug tolerance in pancreatic cancer

Holly Brunton[1], Ludimila Cavalcante[2], Francis Giles[2], Owen Sansom[1], Alexei Vazquez[1]

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2. Actuate Therapeutics, Texas, USA

INTRODUCTION

Pancreatic cancer is profoundly resistant to current therapies. New mechanistic research into the underlying biology of these resistance mechanisms, which can ultimately be translated into more effective therapeutic strategies, is urgently needed. The rapid emergence of resistance in pancreatic cancer is partially accredited to the tumor's ability to mitigate reactive oxygen species (ROS) mediated stress. Enzymes and proteins

central to this detoxification process contain the micronutrient selenocysteine at their active site. Selenocysteine is a cysteine analogue that is more efficient at 'mopping up' free radicals. It also has a unique method of translation, unlike the other 20 amino acids, selenocysteine is not directly encoded by a codon, but rather uses translational recoding, whereby using a specific selenocysteine tRNA it can insert a selenocysteine molecule at a UGA stop codon, consequently hijacking translational termination to produce a selenocysteine containing protein. The specific selenocysteine tRNA uses a unique set of kinases and synthases, known as Phosphoseryl-tRNASec kinase (PSTK), Sep-tRNA:Sec-tRNA synthase (SEPSECS) and Selenophosphate synthetase (SPS), that ready the tRNA for translation. We find that drug persistent pancreatic cancer cells upregulate this pathway to combat therapeutic intervention. Critically, the role of selenocysteine metabolism during drug resistance in pancreatic cancer is unknown. Here we will discuss our latest findings that suggest that selenocysteine metabolism is a novel pathway to resensitize pancreatic cancer to chemotherapy.

MATERIALS/PATIENTS AND METHODS

We interrogated the transcriptome and metabolome of 48 novel pancreatic cancer patient derived cell lines (PDCLs) to define mechanisms of resistance to GSK3B inhibition using a small molecule potent selective GSK3B inhibitor (9-ING-41) with antitumor activity. 9-ING-41 is now in clinical investigation and is currently the subject of phase I/II studies in combination with gemcitabine or gemcitabine plus nab-paclitaxel in patients with metastatic pancreas adenocarcinoma, in some of whom it is generating significant clinical responses.

RESULTS

Redox metabolites increase following targeted therapy in PDAC suggesting that resistant cells are managing to alleviate levels of ROS mediated stress. Despite this elevated stress, proteins that regulate major H2O2 clearing reactions are not transcriptionally upregulated, suggesting an alternative mechanism to mitigate cellular stress. RNA-seq analysis of resistant PDCLs identified that selenocysteine metabolism is significantly altered in resistant PDCLs . Humans have 25 known selenocysteine containing proteins, referred to as selenoproteins, that function mainly as oxidoreductases, where the selenocysteine residue plays a catalytic role in redox regulation and antioxidant activity. After prolonged inhibitor treatment, we observe an increase in O-phosphoseryl-tRNA kinase (PSTK) and Sep-tRNA:Sec-tRNA synthase (SEPSECS) expression, encoding two kinases with essential functions in selenoprotein translation. When cells are grown in the absenceof selenium, translation of selenoproteins terminates at the UGA stop codon, resulting in a truncated non-functional enzyme, suggesting that to increase translation of ROS clearing proteins a cell will require an increased supply of selenium. In support of this hypothesis, resistant cells up-regulate expression of the selenium transporter Solute Carrier Family 26 Member 7 (SLC26A7).

CONCLUSIONS

We hypothesize that targeting selenoprotein translation will reduce the cellular capacity to manage ROS and resensitize PDAC tumours to targeted therapy.

Keywords: pancreatic cancer, selenoproteins, drug resistance, ROS.

Industry talk

The next generation of immunotherapies requires a new generation of cell analysis tools

Pascale Daou
Agilent

Lecture

Preventing cancer metastasis with superoxide scavengers

Pierre Sonveaux
UC Louvain, Belgium

Mitochondrial redox metabolism is central to the life and death of the cell. For example, mitochondrial production of free radicals and subsequent oxidative damage has long been known to contribute to damage in conditions such as ischaemia-reperfusion (IR) injury in stroke and heart attack. More recently mitochondrial redox changes have also been implicated in redox signalling. Over the past years we have developed a series of mitochondria-targeted compounds designed to ameliorate or determine how these changes occur. I will outline some of this work, which suggested that ROS production in IR injury during stroke was mainly coming from complex I. This led us to investigate the mechanism of the ROS production and using a metabolomic approach we found that the ROS production in IR injury came from the accumulation of succinate during ischaemia that then drove mitochondrial ROS production by reverse electron transport at complex I during reperfusion. This surprising mechanism led up to develop further new therapeutic approaches to impact on the damage that mitochondrial ROS do in pathology and also to explore how mitochondrial ROS can act as redox signals. I will discuss how these unexpected mechanisms may lead to redox and metabolic signals from mitochondria in a range of conditions under both healthy and pathological conditions.

Selected talk

Targeting mitochondrial and redox metabolism to prevent relapse in pancreatic cancer

Nadine Abdel Hadi[1], Gabriela Reyes Castellanos[1], Tristan Gicquel[1], Elodie Baudoin[2], Nathalie Auphan Anezin[2], Juan Iovanna[1], Alice Carrier[1]

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2. Centre d'Immunologie de Marseille-Luminy (CIML), Marseille, France

INTRODUCTION

Mitochondria are cell organelles playing a central role in energetic metabolism and cell death. Mitochondrial metabolism is an emerging target in currently refractory cancers such as Pancreatic Ductal AdenoCarcinoma (PDAC) since they are implicated in chemoresistance[1]. To go further, we hypothesize that mitochondrial metabolism is reprogrammed in PDAC tumor and microenvironment cells during therapeutic treatment, supporting the relapse of PDAC patients.

The main objectives are to: (1) Demonstrate that mitochondrial metabolism is reprogrammed during chemotherapy, (2) Identify the molecular and cellular mechanisms underlying this reprogramming, and (3) Use this knowledge to prevent therapeutic relapse.

MATERIALS AND METHODS

We performed *in vivo* assays treating tumor-bearing mice with Gemcitabine alone or in combination with Perhexiline, a mitochondrial Fatty Acid Oxidation inhibitor. We used two different mouse models: (1) xenografts by subcutaneous implantation of human PDAC cells in immunodeficient nude mice, and (2) syngeneic allografts by orthotopic implantation of murine PDAC cells in immunocompetent C57BL/6 mice. Tumor size was measured twice a week with a caliper for the xenograft model and once a week by bioluminescence imaging for the allograft model, and 1-month treatment started when the xenograft reached the size of 200 mm^3 or on day 12 after allograft implantation. The tumors were analyzed at limit point *ex vivo* by flow cytometry after tumor dissociation to measure mitochondrial and redox characteristics: mitochondrial mass, mitochondrial superoxide anions (mtO2.-), mitochondrial membrane potential (MMP) and total reactive oxygen species (ROS). The total ATP level was measured by Cell-Titer Glo. The molecular mechanisms underlying the reprogramming were determined by a transcriptomic analysis (RNA sequencing and RT-qPCR).

RESULTS

In the xenograft model, Gemcitabine treatment induces complete tumor regression for the first xenograft, but the tumors relapse after stopping treatment. The second xenograft behaves differently: Gemcitabine treatment does not induce tumor regression but an arrest of tumor growth, and the combination with Perhexiline induces complete regression followed by relapse after a certain time.

In the allograft model, all tumors regress during Gemcitabine treatment, some completely. But all relapse, sometimes even during the treatment (escape). Interestingly, in both models, relapsing tumors show increased mitochondrial mass and mtO2.- and ROS levels, compared to initial tumors (non-treated tumors), suggesting deregulation of redox control and overproduction of mitochondrial ROS. In the xenograft model only, relapsing tumors exhibit high MMP and total ATP level, suggesting increased energy capacity. Our molecular analysis shows an overexpression of genes encoding antioxidant enzymes in relapsed tumors.

CONCLUSION

Our results show that relapsed PDAC tumors result from the proliferation of persistent/residual cells[2] which survived during therapy-induced regression through the establishment of redox metabolic adaptations. Now, we will determine the molecular and cellular mechanisms underlying this metabolic adaptations in tumor cells, but also in the immune microenvironment which is very abundant in PDAC tumors[3] . To conclude, this work reveals that targeting redox metabolism is a candidate approach to sensitize residual cancer cells to cell death and prevent relapse in PDAC[4].

Keywords: pancreatic ductal adenocarcinomas, energetic metabolism, redox metabolism, mitochondrial metabolism, therapeutic relapse.

References:

1. Masoud, R. *et al. Targeting Mitochondrial complex i overcomes chemoresistance in high OXPHOS pancreatic cancer. Cell Reports Medicine* 1, 100143 (2020).
2. Shen, S., Vagner, S. & Robert, C. *Persistent cancer cells: the deadly survivors. Cell* 183, 860–874 (2020).
3. Roma-Rodrigues, C., Mendes, R., Baptista, P. V. & Fernandes, A. R. *Targeting Tumor microenvironment for cancer therapy. Int J Mol Sci* 20, (2019).
4. Abdel Hadi, N., Reyes-Castellanos, G. & Carrier, A. *Targeting redox metabolism in pancreatic cancer. Int J Mol Sci* 22, (2021).

Industry talk

Nanolive microscopes deliver a complete solution for label-free high resolution live cell imaging and quantitative analysis

Adrian Baumann
Nanolive

Session II
REDOX & HYPOXIA

Chairs: Nathalie Mazure (C3M, Nice, France) **& Marija Vlaski-Lafarge** (EFS/ BMGIC, Bordeaux, France)

Nobel lecture

Understanding cellular oxygen sensing mechanisms: implications for cancer

Sir Peter J. Ratcliffe
Ludwig Institute for Cancer Research, University of Oxford and the Francis Crick Institute, London, United Kingdom

Maintenance of oxygen homeostasis is a fundamental physiological challenge, whilst low oxygen (hypoxia) is an important component of most human diseases. Work which commenced with studies of the oxygen-regulated expression of the erythropoietin gene in the kidneys and liver led to the discovery of a widespread system of oxygen sensing and transcriptional control which operates in essentially all animal cells. This pathway transduces a broad range of cellular and systemic responses to hypoxia, including the regulation of energy metabolism, angiogenesis, erythropoiesis and cell differentiation and survival decisions. The oxygen sensitive signal is generated by a set of oxygen splitting enzymes, which catalyse the post-translational hydroxylation of specific amino acids in the transcription factor hypoxia inducible factor (HIF). HIF prolyl hydroxylation targets HIF-alpha polypeptides for destruction by the von Hippel-Lindau (pVHL) ubiquitin E3 ligase, whilst HIF asparaginyl hydroxylation inhibits co-activator recruitment and reduces transcriptional activation. In hypoxia these processes are suppressed, allowing HIF-alpha to escapade destruction and form an active transcriptional complex. Although the use of post-translational hydroxylation in the regulation of HIF was unprecedented as a signalling mechanism, it is now known that all four eukaryotic kingdoms deploy different types of enzymatically catalysed protein oxidation linking to protein degradation to signal oxygen levels. The lecture will review advances in the understanding of these pathways and consider the implications for cancer and cancer therapeutics.

Pan-genomic analyses of HIF-mediated transcriptional responses has revealed that HIF directly activates thousands of genes in hypoxic cells. Indirect actions of systems that respond to these genes massively amplify the complexity of responses to hypoxia. In cancer hypoxia signalling pathways are dysregulated, both by oncogenic mutations and by the micro-environmental hypoxia that frequently develops in solid tumours. One of the most striking mechanisms of HIF activation in cancer occurs in clear cell renal carcinoma, where biallelic inactivation of the *VHL* gene, encoding the pVHL ubiquitin E3 ligase responsible for

oxygen dependent degradation of HIF-alpha, leads to constitutive upregulation of the HIF pathway even in well-oxygenated cells.

The lecture will consider the implications of 'switching' massively interconnected hypoxia pathways during cancer development and will review evidence for a 'pathway tuning' model of oncogenesis. It will consider evidence that individual components of hypoxia signalling pathways are highly heterogeneous in their effects on cancer growth, involving both pro- and anti-tumorigenic effects and that this generates multiple selective pressures to 'tune' the HIF pathway as VHL-associated kidney cancer develops. Analysis of genome-wide association studies of human polymorphisms associating with VHL-defective kidney cancer reveals striking alignment with cis-acting elements of the HIF transcriptional cascade. This extends even to polymorphisms whose disease-association lies below conventional statistical thresholds. Thus, extremely small genetic differences that appear to act to 'tune' the expression of specific HIF target genes have discernible effects on the development of this type of cancer.
The lecture will discuss the general implications (that very small effects matter in cancer) for oncogenesis and oncology.

Lecture
Breaking the hypoxic barrier to PARP inhibitors

Amato Giaccia[1, 2], Manal Mehibel[1]
1. Department of Radiation Oncology, Stanford University School of Medicine, United Kingdom
2. MRC Institute of Radiation Oncology, University of Oxford, United Kingdom

In this study, we have identified an important mechanism by which the tumor microenvironment, mediated by moderate levels of hypoxia and reduced ROS-mediated DNA damage, promotes PARP inhibitor (PARPi) resistance in homologous recombinant (HR) proficient and HR deficient tumors. This study is important because while most groups have focused on cancer cell intrinsic mechanisms of PARPi resistance, we have identified a mechanism by which the hypoxic tumor microenvironment is responsible for PARPi therapeutic efficacy.

Hypoxia, an important feature of the tumor microenvironment, causes resistance to conventional chemotherapy, but has recently been reported to synergize with PARP inhibitors in HR proficient cells through suppression of HR. While this synergistic killing is true in severe hypoxia, our study shows that moderate hypoxia (2% oxygen) is instead associated with resistance to PARPi in HR proficient as well as HR deficient cells. Mechanistically, we identified reduced ROS-induced DNA damage as the cause for the observed resistance. Taking advantage of a previously established hypoxia gene expression signature and a database of breast PDX models, we found that sensitivity of these tumors to Olaparib was inversely correlated with the level of intratumor hypoxia. To determine the contribution of hypoxia to PARPi resistance, we used the hypoxic cytotoxin Tirapazamine to target hypoxic tumor cells and found that their elimination led to a substantial antitumor response with PARPi compared to PARPi treated tumors alone, without affecting normal tissue toxicity. Our study indicates that hypoxia reduces the efficacy of PARPi and that eliminating hypoxic tumor cells will enhance the efficacy of PARPi therapy.

Our study reveals a new treatment combination by which targeting the hypoxic tumor microenvironment can be exploited to induce PARPi synthetic lethality. Importantly, the hypoxic cytotoxin Tirapazamine can

significantly improve PARPi efficacy without enhancing PARPi normal tissue toxicity. Therefore, we propose the evaluation of Tirapazamine and other hypoxic cytotoxins in combination with PARPi-based therapy in future clinical trials for hypoxic tumors that are unresponsive to PARPi therapy alone.

Industry Symposium
Hypoxia live session

Petra Miikkulainen[1] **& Gaëtan Podeur**[2]
1. Baker Ruskinn
2. Alliance Bio Expertise

Industry talk
Innovative tools for monitoring mitochondrial events in vitro

Frédéric Samazan
Tebu-Bio

Better understanding of the global dynamic biological process requires constant improvement of the robustness and specificity of assays used. For this objective, Tebu-Bio facilitates and regularly expands access to robust and innovative life science reagents, to simplify the scientist's research every day. In this talk, we present a selection of innovative assays developed by Dojindo, to accurately monitor specific mitochondrial events *in vitro*.

Selected talk
Hif1a promotes prostatic intraepithelial neoplasia evolution

Mohamed Abu El Maaty, Celine Keime, Gilles Laverny, Daniel Metzger
Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Illkirch, France

INTRODUCTION

Prostatic intraepithelial neoplasia (PIN) are precancerous lesions that ultimately progress into malignant tumors. Characterizing the temporal evolution of the various cell types in such lesions, as well as the gene expression/regulatory programs that support disease progression, will enable the optimization of chemo-preventive and treatment regimens. Pten(i)pe-/- mice, which harbor a prostatic luminal cell specific deletion of the tumor suppressor Pten at adulthood, faithfully recapitulate disease progression in humans. At 3 months after gene invalidation (AGI), Pten(i)pe-/- prostates contain PINs, which enter a latency phase until 9 months AGI. Between 9 and 12 months AGI, some PINs evolve into adenocarcinoma, and after more than 1 year, all prostates exhibit hybrid focal histologies, involving minor regions of PIN/adenocarcinoma and major regions of poorly differentiated/sarcomatoid carcinoma.

MATERIALS AND METHODS

We conducted longitudinal single-cell RNA-sequencing (scRNA-seq) studies on prostates of genetically-engineered mice at different disease stages: PIN (3 and 6 months AGI), PIN/adenocarcinoma (9 months AGI), and mixed/hybrid malignant tumors (15 months AGI). Further investigations included histological analyses of prostates, immunostaining, flow cytometry, and organoid cultures.

RESULTS

We performed trajectory inference analyses on prostatic luminal epithelial cells of Pten(i)pe-/- mice and identified the activation of HIF1-alpha signaling during disease progression. To study the role of Hif1a in prostate carcinogenesis, we generated Pten/Hif1a(i)pe-/- mice in which both Pten and Hif1a are selectively invalidated in prostatic luminal epithelial cells at adulthood. Single-cell characterization of Pten/Hif1a(i)pe-/- prostates at 3 months AGI demonstrated the down-regulation of glucose-metabolizing networks and modulation of senescence-associated secretory phenotype (SASP) composition in luminal cells. These effects led to a stimulation of immune surveillance in prostates, characterized by a reduction in the infiltration of myeloid-derived suppressor cells (MDSCs) and an increase in the levels of CD8+ T-lymphocytes and natural killer cells, leading to apoptotic induction in some PIN cells. Furthermore, Hif1a invalidation reduced the progression of PINs, and attenuated the development of poorly-differentiated tumors. The expression levels of the pluripotency and plasticity factors Sox2 and Ezh2 were lower in Pten/Hif1a(i)pe-/- tumors compared to Pten(i)pe-/- ones, and epithelial cells with Hif1a ablation had reduced organoid formation capacity. Altogether, our data demonstrate the activation of stemness pathways by Hif1a.

CONCLUSION

We demonstrated that Hif1a signaling is a key driver of PIN progression, by promoting pro-tumoral cell-intrinsic and-extrinsic pathways.

Lecture

The dual metabolic control of stem cell niche: an energy restriction model for the selection of chronic myeloid leukaemia cells sensitive or resistant to therapy

Persio Dello Sbarba

Department of Experimental and Clinical Biomedical Sciences, Università degli Studi di Firenze, Italy

The homing of normal haematopoietic stem cells (HSC) in bone marrow within specific environmental conditions, referred to as the "stem cell niche" (SCN), has been intensively studied over the last three decades. These conditions include the action of a number of molecular and cellular players as well as critical levels of nutrients. The strict analogy between the hierarchical structure of normal haematopoiesis and that of leukaemia cell populations led to propose that leukaemic growth is fostered by cells endowed with stem cell properties, the leukaemia stem cells (LSC), a concept readily extended to that of cancer stem cells (CSC) of solid tumors. Two alternative routes have been proposed for the onset of CSC: the oncogenic staminalization (acquisition of self-renewal) of a normal progenitor cell (the "CSC in progenitor cell" model), or the oncogenic transformation of a normal (self-renewing) stem cell (the "CSC in stem cell" model). The latter mechanism, in the haematological context, makes LSC derive from HSC, implying that LSC most likely share SCN homing with HSC.

We found that incubation in low oxygen, one of the defining physiological features of SCN, of chronic myeloid leukaemia (CML) cells time-dependently suppresses BCR/Ablprotein, the CML driver oncoprotein, but not BCR/AblmRNA. As stem cell potential is not suppressed in low oxygen, an LSC subset apparently exists which is capable to persist independently of BCR/Abl signalling, yet remaining genetically leukaemic. These LSC are refractory to the tyrosine kinase inhibitors (TKi) active on BCR/Abl and used for CML therapy, obviously due to the lack of their molecular target. However, a BCR/Ablprotein-expressing (from now on

referred to as "BCR/Abl-positive") cell subset also exists which exhibits LSC properties, suggesting that in CML the two CSC models we referred to above as alternative should be rather considered complementary. We proposed that BCR/Abl-positive LSC ("LSC in progenitor cell") drive the expansion of leukaemic cell population, whereas BCR/Abl-negative LSC ("LSC in stem cell") are responsible for the long-term maintenance of therapy-resistant minimal residual disease (MRD) of CML. This is a simple, straightforward explanation of the persistent risk of relapse of disease in patients who brilliantly responded to TKi treatment and underwent induction of remission.

An initial characterization of the metabolic mechanisms driving BCR/Ablprotein suppression showed that it occurs when glucose approaches complete exhaustion, which is obviously made easier in a low-oxygen environment (the "Pasteur effect"). We proposed on this basis an SCN model where BCR/Abl-positive or -negative LSC subsets are spatially distributed in low-oxygen tissue sites according to local substrate availability and in function of their different metabolic profile. In SCN zones where glucose is available BCR/Abl expression would predispose CML cells to clonal expansion, whereas zones under glucose shortage would host cells adapted to persist independently of BCR/Abl signaling and thereby to represent a reservoir of treatment-resistant MRD. According to this model, relapse of disease would occur when BCR/Abl-negative LSC adapted to energy shortage, following the establishment of permissive conditions (restored glucose supply), are induced to turn into or generate BCR/Abl-positive LSC capable to sustain clonal expansion.
As glucose shortage does not affect the maintenance of stem cell potential in BCR/Abl-negative CML cells, a key question is which source of energy these cells rely on. CML cells are characterized by a highly glycolysis-oriented baseline metabolic profile, which leads to the release into the environment of abundant lactate. A number of studies carried out with solid cancers showed that lactate released into the environment by the highly glycolytic majority of cancer cells may be taken up as a major energy source (the "metabolic symbiosis") by a minority of cells which is metabolically adapted to take advantage of such a recycle. This led to envision a two-tier model for SCN of CML including: 1) an SCN core where LSC sustaining MRD survive and may cycle and where glucose is absent but other nutrients are available; 2) a highly glycolytic niche periphery (because closer to blood vessels) hosting LSC ready to drive clonal expansion; 3) the diffusion into the core of metabolites generated in the periphery.

The experimental strategy we adopted to deepen the characterization of the two different functional LSC phenotypes of CML is based on the idea that the metabolic compartmentalization of LSC subsets we hypothesized to occur *in vivo* in function of space (distance from blood supply) can be mimicked *via* the progressive exhaustion of nutrients (especially glucose) *in vitro* in function of incubation time. Culture conditions are modulated by adding or subtracting metabolites or inhibitors and cultured cells are analysed to determine the metabolic phenotype, the expression of stem cell markers and the maintenance or loss of stem cell potential. Recent research focused on the role of lactate and glutamine in the conditioning of SCN environment, as well as in the testing of drugs capable to overcome the therapy resistance of LSC.