TRANSMIT
Translating the role of Mitochondria in Tumorigenesis

European Commission

COURSE IN CANCER METABOLISM

Programme
&
Book of abstracts

November 28-29, 2018

TRANSMIT project - Grant Agreement 722605

Contents

Welcome Letter

In 2010 the 1st Course in Mitochondrial Metabolism and Cancer was taking place in Bologna, and paving the way for larger conference series in Europe, at a time when the role of the Mitochondrion and its DNA in cancer was emerging, and research in the field gaining momentum.

The theme of Mitochondria and Cancer would also star during the second Course in Mitochondrial Medicine held in Bertinoro di Romagna: little did we know then that a Marie Curie ITN project would stem from those parentheses, and involve several authoritative Centers in Europe along with 11 brilliant and motivated young researchers.

The TRANSMIT project – TRANSlating the role of Mitochondria in Tumorigenesis – took life during a summer in 2015 and gave us the opportunity to gather here today to discuss the most recent advances in cancer metabolism, with a particular focus on mitochondria in tumor progression.

To those who join us here for the first time, welcome. And to the old friends who reconvene, welcome back once more at the gates of the Italian winter, under the falling amber leaves of the Bertinoro gardens.

Anna Maria Porcelli & Giuseppe Gasparre
(University of Bologna, Italy)

Course in Cancer Metabolism

November 29-30, 2018
CEUB - Bertinoro di Romagna (Italy)

Course Directors

- **Anna Maria Porcelli** (University of Bologna, Italy)
- **Giuseppe Gasparre** (University of Bologna, Italy)
- **Barbara Kofler** (Paracelsus Medical University, Salzburg, Austria)
- **Rodrigue Rossignol** (University of Bordeaux, France)

Faculty Members

- **Ralph J. DeBerardinis** (Children's Medical Center Research Institute, University of Texas Southwestern Medical Center, USA)
- **Fátima Baltazar** (Life and Health Sciences Research Institute, School of Health Sciences, University of Minho, Portugal; ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal)
- **Bernhard Radlwimmer** (Cancer Research Center - DKFZ, Germany)
- **Almut Schulze** (Department of Biochemistry and Molecular Biology, Theodor-Boveri-Institute, Biocenter, Würzburg, Germany)
- **Ana Mateus** (Senior Editor at Nature Metabolism, London, UK)
- **Luigi Ombrato** (The Francis Crick Institute, London, UK)
- **Gyorgy Szabadkai** (Department of Cell and Developmental Biology, Consortium for Mitochondrial Research, University College London, London; Department of Biomedical Sciences, University of Padova, Padova, Italy; The Francis Crick Institute, London, UK
- **Fatima Mechta-Grigoriou** (Stress and Cancer Laboratory, Institut Curie, Inserm U830, Paris, France)
- **Laurent Le Cam** (Institut de Recherche en Cancérologie de Montpellier, INSERM, University of Montpellier, France)
- **Cristina Muñoz Pinedo** (Cell Death Regulation Group, Bellvitge Biomedical Research Institute, Barcelona, Spain)

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TRANSMIT Marie Curie Fellows

ESR1	Saharnaz Sarlak	(University of Bordeaux, France)
ESR2	Ana Carolina B. Sant´Anna-Silva	(OROBOROS Instruments GmbH, Austria)
ESR3	Floriana Jessica Di Paola	(Justus-Liebig University of Giessen, Germany)
ESR4	Christina Schmidt	(University of Cambridge, UK)
ESR5	Nikkitha Umesh Ganesh	(University of Bologna, Italy)
ESR6	Nicole Bezuidenhout	(Karolinska Institute, Sweden)
ESR7	Maheshwor Thapa	(BIOCRATES Life Sciences AG, Austria)
ESR8	Catarina Silva-Almeida	(AvantiCell Science Ltd, Scotland, UK)
ESR9	Houda Abla	(University of Bologna, Italy)
ESR10	Luca Zampieri	(Université catholique de Louvain, Belgium)
ESR11	Daniela Weber	(Paracelsus Medical University, Austria)

Course Programme

Arrival day: November 28 afternoon / evening

Thursday, November 29

Morning Session Chairperson: **Sybille Mazurek** (Justus-Liebig University of Giessen, Germany) **Colin Wilde** (AvantiCell Science Ltd, Scotland, UK)	
08.00 – 09.00	**Registration to the course**
09.00 – 09.10	**Coordinator welcome speech**
09.10 – 10.00	**Keynote Lecture: Metabolic complexity in cancer cells and tumors** Ralph J. DeBerardinis (Advisory board member) *Children's Medical Center Research Institute, University of Texas Southwestern Medical Center, USA*
10.00 – 10.50	**Lactate transporters and pH regulation: potential targets in cancer therapy** Fátima Baltazar *Life and Health Sciences Research Institute, School of Health Sciences, University of Minho, Portugal; ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal*
10.50 – 11.20	**Coffee break**
11.20 – 12.10	**Branched-chain amino acid catabolism in cancer** Bernhard Radlwimmer *German Cancer Research Center (DKFZ), Germany*
12.10 – 13.00	**Targeting cancer metabolism** Almut Schulze *Department of Biochemistry and Molecular Biology, Theodor-Boveri-Institute, Biocenter, Würzburg, Germany*
13.00 – 14.30	**Lunch break**

Afternoon Session I: TRANSMIT ESRs Lectures Chairperson: **Christian Frezza** (University of Cambridge**,** UK) **Maria Shoshan** (Karolinska Institute, Sweden)	
14.30 – 14.50	**Nitrosamine signalling crosstalk with energy metabolism in lung cancer** Saharnaz Sarlak (ESR1) *University of Bordeaux, France*
14.50 – 15.10	**Cellular succinate transport and mitochondrial respiratory function in prostate cancer** Ana Carolina B. Sant´Anna-Silva (ESR2) *OROBOROS Instruments GmbH, Austria*
15.10 – 15.30	**Coordination of glutaminolysis and glycolysis in PC3 prostate cancer cells** Floriana Jessica Di Paola (ESR3) *Justus-Liebig University of Giessen, Germany*
15.30 – 15.50	**Oncometabolic impact of fumarate on tumorigenesis** Christina Schmidt (ESR4) *University of Cambridge, UK*
15.50 – 16.20	**Coffee break**
16.20 – 16.40	**Cancer cell models to test metabolic intervention strategies** Catarina Silva-Almeida (ESR8) *AvantiCell Science Ltd, Scotland, UK*
16.40 – 17.00	**Mitochondrial complex I-driven regulation of the hypoxic response in cancer cells** Nikkitha Umesh (ESR5) *University of Bologna, Italy*
17.00 – 17.20	**PGC1 in chemoresistance and stemness in ovarian cancer** Nicole Bezuidenhout (ESR6) *Karolinska Institute, Sweden*
17.20 – 18.10	**Publishing your work: what you need to know** Ana Mateus (Advisory board member) *Senior Editor at Nature Metabolism, London, UK*

18.10 – 19.10	**Poster viewing session**
19.10 - 20.30	**Regeneration**
20.30	**Dinner**

Friday, November 30

Morning Session Chairperson: **Pierre Sonveaux** (Université catholique de Louvain, Belgium) **Rodrigue Rossignol** (University of Bordeaux, France)	
09.10 – 10.00	**Cancer and its host, a story of corruption** Luigi Ombrato *The Francis Crick Institute, London, UK*
10.00 – 10.50	**Understanding mitochondrial adaptation in cancer by gene expression data** Gyorgy Szabadkai *Department of Cell and Developmental Biology, Consortium for Mitochondrial Research, University College London, London, UK; Department of Biomedical Sciences, University of Padova, Padova, Italy; The Francis Crick Institute, London, UK*
10.50 – 11.20	**Coffee break**
11.20 – 12.10	**Oxidative stress and Metabolic heterogeneity in Cancer** Fatima Mechta-Grigoriou *Stress and Cancer Laboratory, Institut Curie, Inserm U830, Paris, France*
12.10 – 13.00	**Roles of the p53 pathway in metabolism: implications in aging, tissue homeostasis and carcinogenesis** Laurent Le Cam *Institut de Recherche en Cancérologie de Montpellier, INSERM, University of Montpellier, France*
13.00 – 14.30	**Lunch break**

Afternoon Session II: TRANSMIT ESRs Lectures Chairperson: **Guido Dallman** (BIOCRATES Life Sciences AG, Austria) **Barbara Kofler** (Paracelsus Medical University, Salzburg, Austria)	
14.30 – 14.50	**Quantitative analysis of coenzymes and acyl-carnitines in cancer cells** Maheshwor Thapa (ESR7) *BIOCRATES Life Sciences AG, Austria*
14.50 – 15.10	**Investigating αKG derivatives in preventing metabolic adaptation in tumorigenesis: a proof of concept in cancer models** Houda Abla (ESR9) *University of Bologna, Italy*
15.10 – 15.30	**Towards the identification of metabolic changes associated to cisplatin resistance in ovarian cancer** Luca Zampieri (ESR10) *Université catholique de Louvain, Belgium*
15.30 – 15.50	**Ketogenic diet as an adjuvant therapy for melanoma** Daniela Weber (ESR11) *Paracelsus Medical University, Austria*
15.50 – 16.10	**Discussion and conclusions**
16.10 – 16-40	**Coffee break**
16.40 – 17.30	**Closing Lecture: Different forms of cell death induced by inhibition of cancer metabolism** Cristina Muñoz Pinedo *Cell Death Regulation Group, Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain*
17.30 – 18.00	**Best Poster Ceremony**
18.30	**Departure of participants**

Speakers Abstracts

Metabolic complexity in cancer cells and tumors

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Metabolism is dynamic and responds to a wide range of cell-intrinsic and cell-extrinsic factors. Many human diseases involve perturbations of metabolism at the cellular level, and in many cases normalizing the metabolic state has proven to be therapeutically valuable. Cancer is one such disease, in which factors both intrinsic and extrinsic to the malignant cell impact tumor biology and disease progression. In cancer, cell-intrinsic influences on metabolism include somatically-acquired mutations in oncogenes and tumor suppressor genes, many of which regulate metabolic activity. Cell-extrinsic factors include nutrient access, which may become limiting due to inadequate vasculature and intense fuel utilization, and metabolic interactions with stromal and immune cells. A major challenge in cancer metabolism research is to understand how these various factors culminate in the metabolic phenotype of an intact tumor, and ultimately to identify which altered pathways represent potential therapeutic targets. We have taken two parallel approaches to understand metabolic complexity in human cancer. The first uses a combination of multi-parametric imaging and intraoperative stable isotope infusions to assess metabolic fluxes in patients with solid tumors, and to compare fluxes between tumors and adjacent benign tissue. Genomic, histological and metabolic analysis of tumor samples allows us to correlate various intrinsic and extrinsic factors to specific aspects of the metabolic phenotype. The second approach uses standardized culture conditions to assess cell-intrinsic heterogeneity of metabolic preferences and dependencies in large panels of human cancer cell lines. This approach has uncovered liabilities associated with specific molecular subtypes of non-small cell and small cell lung cancer. I will discuss the application of these approaches to metabolic heterogeneity in human cancer, with an emphasis on features relevant to intact tumors and therapeutic liabilities and the role of metabolic imaging in studying cancer metabolism.

Funding:
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Notes

Lactate transporters and pH regulation: potential targets in cancer therapy

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A common feature of cancer cells is a preference for glucose metabolism, in which cells rely on high rates of glycolysis for ATP production, with increased glucose uptake and lactic acid production [1]. This phenomenon was first described by Otto Warburg and is currently known as "aerobic glycolysis" or "Warburg effect". This metabolic phenotype generates high amounts of protons, which leads to acidification of the microenvironment. This acidification has been associated with several features of cancer aggressiveness, including invasion, metastasis, evasion from the immune system, angiogenesis and resistance to therapy [2]. To cope with this, cancer cells rely on the activity of proton exchangers and transporters, which export protons to the microenvironment, allowing cancer cells to survive in this hostile environment. Among these, are monocarboxylate transporters (MCTs), which play a dual role in tumors [3]. On one hand, they allow the maintenance of the glycolytic metabolism by removing lactate from the cell and, on the other hand, they participate in the regulation of pH since they transport lactate through a proton-coupled mechanism.

MCTs belong to the SLC16 family of genes which have presently 14 members, however only the first 4 isoforms (MCT1-4) are known to transport monocarboxylates across cell membranes. The role of MCTs has been studied in several cancer types, including expression in human samples and targeting *in vitro* and *in vivo* [4]. The most important MCT isoforms in cancer are MCT1 and MCT4, which are upregulated in a variety of human malignancies. Additionally, analysis of associations between MCT expression and clinic-pathological data identified MCTs as potential markers of poor survival in some tumor types. Thus, considering their role in cancer, MCTs are currently attractive targets in cancer therapy. Targeting MCT1/4 has been demonstrated to be effective in reducing lactate transport, cancer cell proliferation, invasion and migration and increasing cell death *in vitro*, as well as retarding tumor growth, sensitizing tumor cells to radiation, inducing tumor necrosis and decreasing tumor invasion *in vivo*. Specific MCT inhibitors have been more recently developed by the pharmaceutical industry, which results hold promise, being one of them already in clinical trials.

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References:
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Notes

Branched-chain amino acid catabolism in cancer

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The branched chain amino acids (BCAAs) leucine, isoleucine, and valine largely escape first-pass liver catabolism and are initially transaminated in extrahepatic tissues such as muscle, adipose tissue, pancreas and brain. Metabolism of BCAAs provides an important mechanism by which nitrogen moves throughout the body and its deregulation has been associated with various diseases. The first step in BCAA catabolism is a transfer of an α-amino group to α-ketoglutarate (α-KG) through the activity of cytosolic branched-chain amino acid transaminase 1 (BCAT1) or mitochondrial BCAT2 isoenzymes, yielding glutamate and the respective branched-chain α-ketoacids. We and others showed that overexpression of BCAT1 is essential for sustained cell proliferation in a number of cancers including glioblastoma, mammary carcinoma and myeloid leukemias. Isotope tracing experiments unexpectedly showed that BCAA carbon did not enter the TCA cycle in significant amounts but rather was excreted from the cells in the form of branched-chain keto acids (BCKAs) via the monocarboxylate transporter 1 (MCT1). Evidence also suggests that efflux of BCKAs exerts immunosuppressive effects on tumor-associated macrophages. Furthermore, we could show that knockdown of BCAT1 results in increased intracellular concentrations of alpha-ketoglutarate (αKG) which links the carbon and nitrogen metabolic pathways and can provide information on the metabolic status of cells. αKG is a cofactor for many enzymatic processes including the demethylation of DNA and histones as well as the regulation of HIF1A by prolyl hydroxylases. Indeed, we could show that modulation of BCAT1 expression abolished the stabilization of HIF1A under normoxic conditions and resulted in altered DNA methylation in acute myeloid leukemia and other tumor entities. These data suggest that BCAT1 is controlling tumor-cell phenotype by regulating intracellular αKG level. To visualize changes of intracellular αKG concentrations in living cells we have recently developed novel intramolecular αKG FRET sensors that can be targeted to different cell compartments. In summary, our work is providing further evidence for the eminent role of BCAT1 and αKG in cancer cell biology.

Funding:
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Targeting cancer metabolism

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Enhanced macromolecule biosynthesis is integral to cell growth and proliferation and altered metabolic activity has emerged as one of the hallmarks of cancer. Metabolic reprogramming also enables cancer cells to survive conditions of limited nutrient and oxygen supply that are characteristic for the tumour microenvironment. The enhanced biosynthetic demand caused by rapid proliferation renders cancer cells highly sensitive to perturbation of the metabolic network and provides the rationale for therapeutic targeting of metabolic process. We have applied metabolic profiling and screening techniques to identify specific metabolic vulnerabilities in cancer cells. These studies have provided insight into the essential role for lipid biosynthesis and anti-oxidant generation for growth and survival of cancer cells. These vulnerabilities are particularly prominent under conditions of nutrient and oxygen depletion. We will present recent insight into the importance of lipid metabolism and related processes for cancer cell growth and survival.

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German Research Foundation, German Cancer Aid.

Notes

Nitrosamine signalling crosstalk with energy metabolism in lung cancer

Saharnaz Sarlak, Iris Steuckardt, Benoît Rousseau, Stéphane Claverol, Marc Bonneu, Didier Lacombe, Nivea Dias Amoedo and Rodrigue Rossignol

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Lung cancer is the leading cause of death by cancer worldwide, with non-small cell lung cancer (NSCLC) being the most common histological type. Tobacco smoking is the most important risk factor in the development of lung cancer although the mechanism by which tobacco smoke affects lung cancer development has not been entirely elucidated. In particular, little is known on the impact of tobacco smoke on cancer cells metabolic reprogramming, an emergent hallmark of cancer [1]. Tobacco contains a variety of carcinogens [2] including nitrosamines as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a powerful carcinogen able to induce lung tumors following a single injection in mice. The aim of this PhD project is to study the crosstalk between nitrosamine signaling, metabolic reprogramming and lung tumor biology and bioenergetics. Different models will be used: 1) human lung adenocarcinoma cells (A549) or human lung non-cancer epithelial cells (BEAS2B) exposed to NNK, 2) lung tissue from mice treated with NNK, 3) reconstituted human bronchial epithelium treated or not with NNK, 4) TCGA data from smokers and non-smokers LUAD patients and 5) genetic models of nitrosamine signaling modulation.

Our preliminary findings indicate that 48H exposure to 10µM NNK alters mitochondrial bioenergetics in A549 and BEAS2B cells and similar findings were obtained in the lung of mice treated during 4 days with 50mg/kg NNK/day. The High-Resolution Respirometry (HRR) study of these *in vitro* and *ex vivo* models, respectively, indicated a conserved reduction of the maximal capacity of the oxidative phosphorylation system. Label-free proteomic investigations performed on the treated human cells, reconstituted epithelia and mouse lung tissue further revealed that NNK triggered a reduction in the content of selected mitochondrial proteins. Mechanistic studies are ongoing to discover how NNK interferes with mitochondrial proteome and related bioenergetics function. In particular, we will investigate the effect of NNK-mediated activation of the alpha-7-nicotinic-acetylcholine receptor (α7 nAChR)[3] on energy metabolism using appropriate pharmacological and genetic tools. This PhD research project aims at a better understanding the role of mitochondria in lung cancer initiation, growth and survival.

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Notes

Cellular succinate transport and mitochondrial respiratory function in prostate cancer

Ana Carolina B. Sant´Anna-Silva[1,2], Helmut Klocker[3], Anja Weber[3], Eskil Elmér[4], Andras T. Meszaros[1,2], Erich Gnaiger[1,2]

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Succinate dehydrogenase (SDH, mitochondrial Complex II) links the oxidation of succinate and FAD to fumarate and $FADH_2$ in the tricarboxylic acid (TCA) cycle to electron transfer (ET) from $FADH_2$ to ubiquinone in the ET system. Changes in ET capacity through the succinate pathway affect TCA cycle function and cell respiration [1]. In addition, succinate transmits oncogenic signals from mitochondria to the cytosol by stabilization of hypoxia inducible factor 1α. This, in turn, stimulates the expression of genes involved in angiogenesis and anaerobic metabolism [2], finally enabling tumour progression and metastasis. Succinate uptake is enhanced in various cancer cells [3], and its mitochondrial utilisation is increased in permeabilized prostate cancer cells [4].

To decipher the pathophysiological role of succinate in prostate cancer, we measured the plasma membrane permeability for succinate and utilization of external succinate by mitochondria in terms of succinate pathway capacity and kinetic properties in prostate cancer (multiple metastatic origins) and control cell lines.

Respiration in RWPE-1 (prostate; noncancerous), LNCaP (prostate; lymph node metastasis) and DU145 (prostate; brain metastasis) cells was measured using High-Resolution FluoRespirometry (O2k, Oroboros Instruments) and substrate-uncoupler-inhibitor titration (SUIT) protocols developed specifically for the study. To assess succinate utilization in intact cells independent of a plasma membrane succinate transporter, we applied novel plasma membrane-permeable succinate prodrugs (pS) [5].

In LNCaP cells, transport of external succinate is enhanced through the plasma membrane as compared to the other cell lines, while pS exerted similar effects in all cell lines, suggesting an important regulatory role of the transport mechanism. Furthermore, in LNCaP cells, mitochondria utilize succinate with higher affinity than control cells. Importantly, kinetic measurements demonstrated the most pronounced difference in the affinities in the physiological intracellular succinate concentration range (< 100 µM), underlining its pathophysiological role.

Our results indicate a “succinate phenotype” in LNCaP, with enhanced transport and utilization. As such, succinate is a potential mitochondrial metabolic biomarker in prostate cancer cells. We propose a model in which succinate does not only play a role in the signalling but has a central role in the maintenance of mitochondrial respiration as a fuel substrate.

Acknowledgements
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References:

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Coordination of glutaminolysis and glycolysis in PC3 prostate cancer cells

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Cancer cell proliferation strongly depends on the availability of energy as well as metabolic precursors for the synthesis of cell building blocks, which implies a close coordination between the associated key pathways: cytosolic glycolysis and mitochondrial glutaminolysis. In glycolysis, tumor cells express a certain pyruvate kinase isoenzyme termed pyruvate kinase M2 (M2-PK, PKM2). In tumor cells the tetramer : dimer ratio of PKM2 regulates whether glucose is converted to pyruvate and lactate with regeneration of energy (highly active tetrameric form) or channeled into synthetic processes (nearly inactive dimeric form). In different cell culture studies an increase in intracellular ROS concentrations induced by insulin treatment of the cells, addition of H_2O_2 into the cultivation medium or hypoxia led to cysteine oxidation of the PKM2 protein, subunit dissociation of PKM2 and a decrease of PK activity which resulted in a decrease in glucose consumption and lactate production. Several studies suggest that hypoxic conditions cause increased ROS production by mitochondrial complex III. During hypoxia myxothiazol, which inhibits site III_{Q0}, was shown to decrease ROS production and to block HIF-1α, whereas antimycin A, which induces superoxide production from side III_{Q0}, was characterized to increase ROS production and HIF1α. Besides mitochondrial complex III also mitochondrial glycerol 3-P dehydrogenase (mG3PDH) has been shown to participate in mitochondrial ROS production to both the mitochondrial matrix as well as the intermembrane space and cytosol.

In our project we investigate the effect of mitochondrial complex III inhibition by antimycin A and myxothiazol as well as of mG3PDH inhibition on PKM2, the composition of the glycolytic enzyme complex, the isoenzyme equipment of lactate dehydrogenase and of the malate aspartate shuttle enzymes malate dehydrogenase and glutamate oxaloacetate transaminase as well as the coordination of glycolytic, glutaminolytic and serine conversion rates in PC3 prostate cancer cells at both 21% oxygen as well as 1.5% oxygen cultivation conditions. The impact of antimycin A, myxothiazol and mG3PDH inhibitors iGP1 and RH02211 on cell proliferation and the metabolic flux rates will be presented.

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Cancer cell models to test metabolic intervention strategies

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Cancer is a complex disease affecting more than 3.7 million persons every year and representing the second highest cause of death and morbidity in Europe (WHO, 2017). In order to survive and proliferate, cancer cells must acquire several biological features, also named “hallmarks of cancer”. These hallmarks include: resisting cell death, inducing angiogenesis, enabling replicative immortality, activating invasion and metastasis, evading growth suppressors and sustaining proliferative signaling [1]. Increasing research has now demonstrated that cancer cells must adjust their metabolism to be more invasive and increase proliferation. Besides the 6 mentioned hallmarks, metabolic reprogramming has also become an additional hallmark of cancer [2]. Recent evidences place mitochondria as a key mediator of this metabolic plasticity. Besides the fact that mitochondria have a crucial role in energy production, mutations in metabolic enzymes (encoded by mitochondrial DNA) are also associated to different types of cancer. Moreover, mitochondria are also targets of oncogenic transduction pathways [3]. For all these reasons, it has become obvious that mitochondria and metabolic reprogramming are important hallmarks in cancer and should be exploited with the intent to discover novel anti-cancer therapies.

The study of cancer biochemistry has been traditionally done using cell lines of various lineages and phenotype. However, many times their relevance to the study of specific cancer types is doubtful. The alternative is to use primary cancer cells, isolated from human tissue and cultured to maintain the initial molecular and functional characteristics. Preclinical models using these cells often produce high predictive value and can act as better predictors of success in clinical trials [4]. ACS has cell-based technologies to create novel *in vitro* models that display the aberrations in cancer cell metabolism which are responsible for disease progression. The models used in the project originate from samples of human tissue obtained with full ethical permission from patients undergoing medically-prescribed surgery. The characteristics of tumor cells are defined by anonymized donor information and selective metabolic analysis. Cells are characterized either in 2D culture or in 3D customized structures, with enhanced physiological relevance and analytical predictive value. The 3D cultures are assembled by use of a defined extracellular matrix or by additive printing of scaffold and cancer cells in defined architectures [5]. The aim of this study is to create a novel primary cell-based 3D platform to test metabolic therapeutic intervention. The model should be readily translatable to "plug and play" format.

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Notes

Oncometabolic impact of fumarate on tumorigenesis

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Renal cell carcinoma (RCC) is amongst the ten most common cancers in the world[1]. Recent work classified RCCs tumour into seven molecular subtypes, associated with mutations of established cancer gene drivers[2]. Among these genes, the mitochondrial enzyme Fumarate Hydratase (FH), when mutated, leads to Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC), a cancer predisposition syndrome characterised by a very severe and agressive form of RCC associated with early metastasis and poor clinical outcome[3,4].

FH catalyses the hydration of fumarate to malate[5]. Consequently, fumarate aberrantly accumulates in FH-deficient cells, reaching high millimolar levels[6]. Several lines of evidence indicate that fumarate contributes to RCC tumorigenesis[7]. For instance, fumarate accumulation causes a post-translational modification of proteins called succination, leading to the upregulation of the antioxidant gene NRF2[8,9]. Fumarate accumulation can also increase angiogenesis and cell growth *via* Hif1α stabilisation[10]. Finally, we recently showed that high fumarate levels trigger an epithelial-to-mesenchymal transition (EMT) through the epigenetic suppression on antimetastatic miRNA, miR200[11]. Yet, how these different signalling cascades propagate upon FH loss and drive transformation is currently unknown.

Here, we capitalise on a novel FH-deficient model to elucidate the oncometabolic impact of FH loss and fumarate accumulation in human cells. Using CRISPR/Cas9-based genome editing, we generated FH-deficient HK2 cells, an immortalized proximal tubule cell line obtained from normal adult human kidney. We showed that this model faithfully recapitulates the biochemical and phenotipic features of FH loss that we have previously characterised, including increased intracellular fumarate concentration, higher glycolytic flux, and mitochondrial dysfunction. Moreover, we found elevated levels of the adduct 2-succino-cysteine, confirming the increased succination, and expression of NRF2 target genes. Importantly, in these cells, FH loss induces the suppression of E-Cadherin and Epcam, hallmarks of EMT. Our future goal is to use this model to capture how these signals propagate over time to elucidate their contribution to cell transformation.

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MRC Cancer Unit Cambridge

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Notes

Mitochondrial complex I-driven regulation of the hypoxic response in cancer cells

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Targeting respiratory complex I (CI) has shown to be a promising anti-cancer strategy. In particular, CI inhibitor metformin has given encouraging results in experimental and clinical settings for the treatment of solid cancers. In recent years, the anti-tumorigenic behavior of CI-deficient tumors has been associated, among others, with HIF-1α destabilization, but the mechanisms behind this association remain unclear. Moreover, even though they are less aggressive, CI-deficient tumors still persist. Our aims were (i) to prove that targeting CI arrests tumor growth by converting them into benign oncocytomas (ii) to investigate the alternative mechanisms that the CI-deficient tumors activate to progress (iii) to seek adjuvant therapies against such mechanisms to improve the CI-targeting effect.

CI-deficient osteosarcoma 143B and colorectal HCT116 cancer cells, lacking CI via knock-out of nuclear-encoded CI core subunit NDUFS3, were used. Retro-X Tet-Off Advanced Inducible Expression system was used to create a stable transgenic cell line that re-expresses NDUFS3 and allowed its inducible knock-out. Tumorigenic potential was evaluated in CD1 nude mice in vivo. The xenografts were analyzed by immunohistochemistry for HIF-1α, vessel markers, MIF (Macrophage migration inhibitory factor) and TAMs. qRT-PCR was used to evaluate the expression of HIF1-responsive genes and MIF.

The lack of NDUFS3 caused a significant decrease of xenograft growth in both 143B-/-163 and HCT-/- tumors. Using the Tet-Off /DOX inducible system we established that targeting CI during cancer progression converts aggressive carcinomas into low-proliferative, oncocytoma-like tumors. Furthermore, CI-deficient tumors were characterized by lack of HIF-1α stabilization and small, lumen-free and SMA-negative vessels. Indeed, compared to their CI-competent controls, CI-deficient tumors revealed a higher content of pro-tumorigenic M2 tumor associated macrophages (TAMs) in association with downregulated MIF expression. Targeting CI in mice through metformin in combination with TAM inhibitors (clodronate/PLX), impaired tumor growth significantly, showing a synergistic effect of the drugs.

CI-deficient tumors strive by triggering non cell-autonomous mechanisms, independent of HIF-1α. This was indeed supported by the down regulation of MIF in our CI-deficient models. Since MIF is a HIF-1α induced pro tumorigenic cytokine, we suggest that the M2 macrophage recruitment we observe in CI-deficient cancers may occur following the disruption in the MIF-HIF-1α axis.

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Notes

PGC1 in chemoresistance and stemness in ovarian cancer

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Tumour-initiating cells (TICs) or cancer stem cells (CSC), have been identified in many malignancies and are believed to underlie recurrent and chemoresistant disease. They are characterized by the expression of stem cell markers, high chemoresistance and an oxidative/mitochondrial metabolic profile in response to microenvironmental challenges [1]. If and how these characteristics are connected remains largely unclear. Earlier data from our lab, using our own SKOV-3 ovarian cancer cell model, consisting of parental SKOV-3 (P) and the cisplatin –resistant SKOV-3 (R) cells, showed that R cells completely lack expression of PGC1α and display TIC properties such as expression of TIC markers CD117 and ALDH1A, and form spheres in stem cell medium [2]. We have aimed to investigate the metabolic/mitchondrial properties of R cells, and how these as well as the PGC1 isoforms relate to cisplatin resistance and stemness.

Results indicate that R cells are able to resist glucose deprivation, and display higher ATP/ADP ratios under basal conditions, as well as under glutamine deprivation, and inhibition of the electron transfer system and glycolysis. Furthermore, P and R cells display differences in fatty acid metabolism and these properties are being further investigated for involvement in chemoresistance and possible interaction with PGC1 isoforms. While R cells completely lack PGC1α, P and R cells express similar levels of the PGC1β isoform. siRNA-mediated knockdown of PGC1α in P cells reduced TFAM expression but did not affect CD117 and ALDH1A expression. Importantly, the PGC1α knockdown significantly increased cisplatin resistance in P cells. siRNA-mediated knockdown of PGC1β in P and R cells showed no effect on cisplatin responses or CD117/ALDH1A. We suggest that PGC1β has overlapping functions to those of PGC1α such as maintaining mitochondrial biogenesis, and that PGC1α has a unique function in cisplatin sensitivity.

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Notes

Publishing your work: what you need to know

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In this talk I will explain the editorial process at Nature journals. In particular, I will highlight the differences between the several journals, which is relevant when choosing where to submit manuscripts. I will then explain how editors assess papers and decide whether to send to peer-revew and also provide some advice into how to improve the manuscript before submission. Finally, I will address the peer-review process and how editors evaluate the reviewer reports and revisions. I will also take the opportunity to introduce Nature Metabolism, a recent addition to Nature-branded research journals, dedicated to the disciplines of metabolism and physiology.

Notes

Cancer and its host, a story of corruption

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The tumour microenvironment or niche is the vital non-cancerous compartment of the tumour structure. Tumour cells and their microenvironment establish a synergistic cooperation that characterizes all aspects of tumour growth, from onset to metastasis. Importantly, this ever-changing interaction is a key source of cancer cells' plasticity and plays an important role in every aspect of tumour progression (1) (2). To date, a direct investigation of the early cellular changes induced by metastatic cells in the surrounding tissue in vivo is difficult to achieve, especially at early micro-metastatic stages. We developed a strategy whereby tissue infiltrating cancer cells label neighbouring cells in vivo and allows direct identification of metastatic niche cells within the whole tissue, to define changes in the local micro-metastatic niche. We uncovered a remarkable local lung epithelial regenerative response, where lung alveolar epithelial cells in the niche show stem-cell features with multi-lineage differentiation potential. We here show that the perturbed lung epithelial cells fuel cancer cell growth, which is further enhanced by changes in niche myeloid cells and highlight the radical tissue local remodelling accompanying cancer cell growth.

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Notes

Understanding mitochondrial adaptation in cancer by gene expression data

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Cancer development and evolution reflects adaptation to signals from oncogenes and the tumour environment. Metabolic adaptation, supporting co-evolution of cancer and stromal cells is presumed to play a key role in tumour development, but how this adaptation occurs at the global cellular level is mostly unknown. The lecture will focus on the use of a novel bioinformatic method (MCbiclust)[1] to detect massive correlated gene and sample biclusters that stratifies breast cancer in different metabolic subtypes based on large unique patterns of nuclear encoded mitochondrial gene expression, and how this can reveal correlating patterns of all cellular metabolic gene expression. These expression patterns define metabolic behaviour of cells, as can be measured by mass spectrometry of 13C carbon fluxes from different substrates. The analyzed modules of central carbon metabolism show specific regulation, each associated with adaptive mitochondrial function. I will describe in detail glutamate-secreting phenotype of invasive lobular carcinoma, co-adapted with low mitochondrial function and suppressed mitochondrial biogenesis, indicating specific gene regulation. Importantly, this metabolic genotype correlates with epithelial-mesenchymal transition, and the large scale metabolic and phenotypic gene pattern regulation is determined by miRNA and gene methylation patterns. Finally, I will discuss how this metabolic gene expression profiling stratifies breast cancer into groups capable of predictions for overall patient survival, and can be developed as biomarkers.

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Notes

Oxidative stress and metabolic heterogeneity in cancer

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In last years, we demonstrated that a chronic oxidative stress accelerates aging (Laurent, *Cell Metabolism*, 2008) and increases metastatic spread, by deeply modifying **tumour micro-environment** (Toullec, *EMBO Mol Med*, 2010; Costa, *Semin Cancer Biol,* 2014; Lefort, *Oncogene*, 2017). However, while oxidative stress increases tumour development, it can also **improve response to chemotherapy** in ovarian cancer patients, in particular to Taxanes (Mateescu, *Nature Medicine*, 2011; Batista, *IJBCB*, 2014; Batista, *Nat Commun,* 2016). Moreover, **autophagy**, cellular process well-known to circumvent intracellular stresses, helps tumour cells from triple-negative breast cancer patients to escape from chemotherapy (Lefort, *Autophagy*, 2014). Thus, oxidative stress promotes tumorigenesis but can, in the mean-time, improve chemosensitivity, defining the paradoxical effect of Reactive Oxygen Species. Altogether, our data have deciphered the pros and cons of intracellular oxidative stress and activation of downstream signalling pathways, such as MEK/ERK, with immediate translational applications for cancer patients (Gruosso, *Nat Commun*, 2015; Gruosso, *EMBO Mol Med*, 2016). Finally, in the last years, using various approaches including flow cytometry on fresh human tissues, immunohistochemistry combined to automated image analysis and functional assays, we have identified **4 different subpopulations of carcinoma-associated fibroblasts** in human breast and ovarian carcinomas, with immunosuppressive functions (Givel, Nat Commun 2018; Costa, Cancer Cell, 2018).

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Notes

Roles of the p53 pathway in metabolism: implications in aging, tissue homeostasis and carcinogenesis

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The p53 pathway is one of the most commonly deregulated tumor suppressor pathways in cancer. The p53 transcription factor controls an efficient safeguard mechanism that prevents cancer progression by regulating DNA repair, cell cycle progression, cell death or senescence. However, its tumor suppressive functions have recently been extended to the control of cellular metabolism. Our projects aim at characterizing new regulatory mechanisms of the p53 pathway, including those implicating the multifunctional protein E4F1 and the MDM2 oncoprotein. In the past years, we identified atypical functions of those important regulators of the p53 pathway in metabolism. Interestingly, although E4F1 and MDM2 were initially characterized as E3 ligases that regulate p53 at the post-translational level, we found that they control, at the transcriptional level, metabolic genes and control mitochondrial activities. Thus, p53, together with its upstream regulators E4F1 and MDM2, define a complex metabolic network that influences both normal tissue homeostasis and cancer progression. Using complementary approaches including the generation of genetically engineer mouse models, metabolomics, and computational modeling, we are trying to define the role of these metabolic networks on stem cell maintenance and aging. In collaboration with clinicians and pathologists, we also investigate the relevance of these new regulatory mechanisms of the p53 pathway with human tumorigenesis, with a particular interest in skin cancers and liposarcomas.

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Notes

Quantitative analysis of coenzymes and acyl-carnitines in cancer cells

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The Warburg paradigm, a prototype model for cancer metabolism, observed that the cancer cells consume glucose to lactate in presence of oxygen. This observation led him to purpose that the respiration defects are the underlying cause for cancer. However, recent studies suggest that mitochondrial metabolism is essential for tumorigenesis and a variety of fuels other than glucose also support for their proliferation and survival. Hence, inhibition of mitochondrial metabolism is emerging as a potential therapeutic strategy for cancer treatment. As coenzymes and acyl-carnitines play a major role in mitochondrial function, their analysis could improve the understanding of mitochondrial dysfunction and facilitate the identification for new therapeutic targets. However, a precise and accurate quantitative analysis of these metabolites is highly challenging task due to their low intracellular concentrations and high instability.

A new simple and sensitive assay method will be developed using liquid chromatography tandem mass spectrometry. Altogether acyl-coenzymes (C0-C20), acyl-carnitines (C0-C18) and other coenzymes like ATP, ADP, NAD, NADH, NADP and NADPH are enlisted for the method development. The novel liquid chromatography-based separation method will be carried out to achieve optimal separation and quantification for all targeted metabolites. The developed method will be validated according to US Food and Drug Administration (FDA) validation guidelines and will be applied to different cancer cells. Cancer cell culture, metabolic quenching and metabolite extraction methods will be optimized to ensure the true metabolic state of the cells at the time of harvesting. Univariate as well as multivariate analysis will be carried out to investigate the metabolite profiling of normal and cancer cell.

We expect the development of fit-for-purpose assay for the analysis of coenzymes and acyl-carnitines in cell culture samples using LC-MS/MS. Further the developed method will be applied to different cancel cell lines to get new insights in the importance of mitochondrial function to cancer development. Coenzymes role will be assessed based on the change in concentrations of coenzymes in different cells types.

A new simple and sensitive assay method will be developed for the measurement of coenzymes and acyl-carnitines and applied to different cancer cells to get the better understanding of mitochondrial function and dysfunction to cancer development.

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Notes

Investigating αKG derivatives in preventing metabolic adaptation in tumorigenesis: a proof of concept in cancer models

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Hypoxia Inducible Factor-1α (HIF-1α) is a transcription factor that once activated controls the transcription of target genes implicated in glycolysis, angiogenesis and survival. To sustain their high growth rate, solid tumours are characterized by a specific metabolic phenotype mainly orchestrated by HIF-1α, which expression is regulated by O2 levels, Prolyl Hydroxylases (PHDs), enzymes responsible of its destabilization as well as TCA cycle intermediates α-ketoglutarate (α-KG)/succinate (SA) ratio. Interestingly, previous works linked mitochondrial complex I absence or deficiency to α-KG accumulation and a failure to adapt to hypoxia that overall correlated with cancer cells reduced tumorigenic potential (Calabrese et al., 2013). In this frame, this project seeks to prevent the metabolic switch and impinge on cancer progression by increasing α-KG intracellular levels to provoke PHDs-mediated degradation of HIF-1α through the screening of selected cell permeable α-KG ester derivatives. Moreover, to mimic nutrients and oxygen restriction conditions, the best compounds effects will be investigated on 3D spheroids as well as on Drosophila melanogaster cancer models with a final aim to develop new therapeutics to associate to conventional treatments.

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Notes

Towards the identification of metabolic changes associated to cisplatin resistance in ovarian cancer

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Chemotherapy is a main treatment modality for cancer. However, acquired chemoresistance can impair clinical outcome. Cisplatin is an effective first line treatment in ovarian cancer, however this therapy often results in cisplatin-resistant recurrences. Studying a cisplatin-resistant model, SKOV-3-R, this project aims to identify metabolic changes associated to cisplatin resistance in advanced ovarian cancer. The focus is set on mitochondrial metabolism. If metabolic changes related to cisplatin resistance were identified, we would further investigate preclinically adjuvant strategies capable of restoring chemosensitivity, thus preventing tumor resistance, relapse, progression and metastasis.

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Notes

Ketogenic diet as an adjuvant therapy for melanoma

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The ketogenic diet (KD) - a high fat, low carbohydrate and adequate protein diet - potentially targets the Warburg effect, a biochemical phenomenon in which cancer cells predominantly utilize glycolysis instead of oxidative phosphorylation (OXPHOS) for energy production. Some types of cancer have low OXPHOS activity and lack the ability to metabolize ketone bodies. Thus, the rationale in providing a KD as adjuvant cancer therapy is to reduce circulating glucose, and consequently insulin levels, and to induce ketosis in such a way that cancer cells will be starved of energy while normal cells will survive [1, 2]. Recently, it has been shown that neuroblastoma and other tumors with high glycolytic activity can be successfully targeted by the KD [3-5]. The aim of the present study was to determine the effect of the KD on melanomas with different genetic alterations.

Human BRAF/NRAS/NF1 wild-type (triple-WT) and BRAF V600E mutated (BRAF V600E) melanoma xenografts were established in CD1 nu/nu mice with WM3311 and WM47 cells, respectively. The melanoma-bearing mice were fed with a standard diet (SD) and different KDs (based on long-chain triglycerides only or supplemented with medium-chain triglycerides). We evaluated the effects of the KDs on tumor growth, body weight, blood parameters such as β-hydroxybutyrate and glucose levels, and liver inflammatory parameters.

KDs significantly decelerated the growth of triple-WT and BRAF V600E melanoma xenografts. Moreover, the long-chain triglyceride-based KD significantly increased the survival of triple-WT melanoma bearing mice compared to mice fed with SD. As expected, KDs significantly increased the level of ketosis and decreased blood glucose levels of both triple-WT and BRAF V600E melanoma bearing mice. Previously, we have shown that KDs can be deleterious in the treatment of renal cell carcinoma by provoking a raise of interleukin-6 (IL-6) and C-reactive protein (CRP) expression in the liver [6]. However, IL-6 and CRP were not altered in triple-WT melanoma bearing xenografts by KDs compared to SD fed mice, indicating that a KD does not induce liver toxicity during the treatment of triple-WT melanoma.

Our preliminary data indicate that KDs could be considered as an adjuvant therapy of triple-WT and BRAF V600E melanoma.

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Notes

Different forms of cell death induced by inhibition of cancer metabolism

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The final desired outcome of therapies targeting the abnormal metabolism of cancer cells is to induce their death. Upon transient nutrient deprivation or treatment with anti-metabolic drugs, cancer cells are frequently able to resolve metabolic stress and survive. However, sustained energy/nutritional stress or prolonged treatment with drugs that inhibit metabolism leads to cell death.

Apoptosis is the best characterized form of cell death. Two main pathways for induction of apoptosis exist: the Death Receptor and the Mitochondrial pathway. Data from our group and numerous other groups indicate that both forms can be induced by metabolic stress induced by nutrient starvation (1-3). Most commonly, anti-metabolic drugs targeting glycolysis or other metabolic pathways kill via induction or activation of BH3 proteins of the Bcl-2 family, which engage the mitochondrial pathway (4).

Classically, non-apoptotic cell death has been classified as necrosis, and many instances of nutrient deprivation and ischemia cause necrotic cell death. In recent years, researchers have characterized some subroutines of necrosis with a certain degree of biochemical detail and have identified proteins that mediate these non-apoptotic forms of cell death (5). I will discuss necroptosis, ferroptosis, pyroptosis, entosis and some examples of involvement of these forms of cell death in the response to ischemia, nutrient deprivation and treatment with anti-metabolic drugs.

Regarding the stress response pathways that engage cell death, it has been traditionally thought that the loss of ATP, followed by activation of the AMPK/mTOR pathway, was responsible for triggering apoptosis. Currently, however, the Integrated Stress Response orchestrated by eIF2α kinases and the transcription factor ATF4 -engaged by endoplasmic reticulum stress or by amino-acid depletion- has emerged as the major signaling pathway that promotes apoptosis or necrosis (6).

I will additionally discuss other links of metabolism and the cell death machinery that suggest that anti-metabolic therapy may be a useful combination with other therapies that promote cancer cell death.

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Notes

Posters Abstracts

P1. Metabolic characterization of cancer stem cells derived by PDAC cell lines

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies with a 5-years survival rate lower than 10%. It is characterized by rapid progression, therapy resistance, and high propensity for metastatic spread.

Emerging evidences suggest that the capacity of a tumor to grow and propagate is mostly dependent on a small subset of cells, known as cancer stem cells (CSCs), which play a pivotal role in tumorigenesis, tumor heterogeneity, radio/chemo-resistance, metastasis, and relapse. For these reasons, we have characterized pancreatic CSCs in order to identify their specific features that may represent new potential targets for therapies.

We obtained CSCs from different PDAC cell lines, focusing particularly on Panc-1, by using a selective culture medium. We observed that CSCs maintained in culture for long time undergo morphological changes. At 2 weeks of culture, they form suspension aggregates and, after an intermediate phase at around 4 weeks, they form spheres with well-defined edges at 8 weeks of culture. We found a progressive increase of the main stem and mesenchymal marker expression levels and of self-renewal capability in CSCs at increasing culture times, also in comparison to parental cells. In line with the acquisition of stem-cell features, proliferation rate experiments show that all CSCs (2-4-8 weeks) grow more slowly than the parental cells and, in particular, CSCs at 8 weeks display a doubling time significantly higher than the other CSCs. Furthermore, cell cycle distribution analysis show that, with the increase of culture weeks, CSCs progressively enhance the percentage of cells in G0/G1 to the detriment of the G2 phase and this shift is significant between parental cells and CSCs at 8 weeks.

In order to study the metabolic features of CSCs, we performed metabolomic analysis and oxygen consumption rate assay. Preliminary data suggest that, in comparison to parental cells, CSCs cultured for 2 weeks show a higher glycolytic metabolism, which shifts to a more oxidative metabolism after 4 weeks of culture. Interestingly, long cultured CSCs enter in a quiescent state, without having senescence markers.

Altogether the described data strongly suggest that long culture times determine the acquisition of marked stem features.

P2. Cross-talk between metabolic and signaling features in the context of T-Acute Lymphoblastic Leukemia cells

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T-cell Acute Lymphoblastic Leukemia (T-ALL) is an aggressive hematological malignancy, still poorly characterized from a molecular point of view, in which T-cell precursors transformation involves the cooperative effect of key genes alterations, including Notch1 constitutive activation and loss of PTEN. Genetic mutations together with interrelated signaling deregulation, such as PI3K/Akt/mTOR pathway overexpression, can lead to a metabolic reprogramming.

Previous studies performed in our laboratory demonstrated the efficacy of the combined targeting of cellular metabolism and PI3K/Akt/mTOR signaling in the context of primary effusion lymphoma. Thus, based on these recent findings and on a broad characterization of T-ALL cells, the aim of our study is to tackle metabolic features to be exploited for novel therapeutic protocols for T-ALL.

We examined the effect on cell viability and proliferation of either acute or chronic exposure to the glucose analog/hexokinase inhibitor 2-DG, the dual PI3K/mTOR inhibitor PF-4691502 and the CK2 inhibitor CX-4945, as single or combined treatment, of a panel of 13 well characterized T-ALL cell lines recapitulating the hallmarks of the disease.

We found that cells carrying both Notch1 and PTEN mutations not only displayed a more glycolytic phenotype, compared to those with wild type and/or a single mutation, but showed also a higher OXPHOS activity, assessed by means of the Seahorse XF96 Analyzer, indicating that the prominent glycolytic rate does not depend on mitochondrial dysfunction. Besides, in these cells combined blockade of glycolysis by 2-DG and PI3K/mTOR signaling by PF-4691502 displayed synergistic cytotoxicity, but it also dropped the extracellular acidification rate (ECAR) as well as the oxygen consumption rate (OCR), and therefore rendered these cells metabolically inactive. Conversely, cells carrying wild type Notch1 and PTEN exhibited a lower metabolic activity. Here the association of 2-DG and PF-4691502 triggered apoptosis to a lesser degree. By further investigation we found that these cells display high levels of PTEN phosphorylated at S380, an inhibitory site phosphorylated by CK2, leading to constitutive PI3K/mTOR activation. Eventually, we demonstrated that cell treatment with the specific CK2 inhibitor CX-4945, alone or combined to 2-DG, overcome aberrant PI3K/mTOR signaling leading to extensive cytotoxicity.

P3. Mutant p53 proteins trigger chemoresistance stabilizing GAPDH in the cytoplasm of pancreatic cancer cells

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Metabolic alterations are a hallmark of tumour cells and provide a key contributor to tumour development *(1).* Tumorigenesis is dependent on the reprogramming of energetic metabolism as consequence of oncogenic mutations. The *TP53* tumor suppressor gene is mutated in ~70% of pancreatic adenocarcinomas (PDAC) generally causing conformational changes in mutp53 proteins triggering proliferation and aggressiveness of cancers through the stimulation of cell cycle, genomic instability, chemoresistance, invasion, metastasis and the counteraction of apoptosis and cellular senescence *(2,3).* This project shows aberrant metabolic changes induced by mutant p53, demonstrating a novel functional link between mutp53 and the subcellular distribution of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a multifunctional and pleiotropic enzyme whose role mainly depends on its cellular localization. We functionally demonstrated that mutp53 proteins inhibit the nuclear translocation of GAPDH stabilizing its cytoplasmic localization, thus supporting the aerobic glycolysis pathway, also named the Warburg effect, and preventing cell death mechanisms mediated by nuclear GAPDH. We further studied the mechanisms by which GAPDH is stabilized by mutp53 in the cytosol. A mechanism by which mutp53 prevents GAPDH nuclear translocation is the stimulation of Akt/mTOR pathway and the repression AMPK pathway, thereby determining a conformational modification of GAPDH undergoing phosphorylation. Moreover, since Sirt1 is known to bind GAPDH inhibiting its nuclear translocation, we discovered that mutp53 stabilizes GAPDH in the cytosol also through the stimulation of GAPDH-Sirt1 protein-protein interaction. By using a specific siRNA-GAPDH or conformational inhibitors of the enzyme, we functionally demonstrated that the stabilization of GAPDH in the cytosol of cancer cells mediated by mutp53 has proliferative, anti-apoptotic and anti-autophagic effects. Furthermore, the blockage of its mutp53-dependent cytoplasmic stabilization is able to restore the sensitivity of PDAC cells to gemcitabine drug. We also show that mutp53 determines an enhanced sensitivity to the stamdard glycolytic inhibitor 2-deoxy-D-glucose. This suggests the triggering of nuclear GAPDH as a potential personalized therapeutic approach in cancers carrying mutant *TP53* gene and highlight the importance of glycolytic drugs as a therapeutic approach in cancers carrying mutant *TP53* gene.

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P4. IDH1 is a pro-senescent therapy in cyclin E-altered HGSC

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Epithelial ovarian cancer (EOC) is the most lethal gynecological cancer. High-grade serous carcinoma (HGSC) is the most fatal histosubtype of EOC. We found that HGSC cell lines with high cyclin E exhibit increased TCA cycle metabolism compared to fallopian tube cells. Therefore, we propose that inhibition of TCA cycle metabolism may be a novel therapeutic strategy for these patients. To determine which TCA cycle enzymes are dysregulated, we performed an unbiased qPCR screen. Our results indicate that isocitrate dehydrogenase I (*IDH1*) expression is significantly altered in cyclin E-altered HGSC cell lines compared to fallopian tube cells. IDH1 catalyzes the conversion of isocitrate to alpha ketoglutarate (aKG). IDH1 mutations are known to play a role in cancer; however, recent publications suggest wildtype *IDH1* promotes primary glioblastoma progression. Wildtype IDH1 and its role in metabolism has never been investigated in cyclin E-altered HGSC.

To determine whether IDH1 plays a role in HGSC, we knocked down IDH1 in multiple cyclin Ealtered HGSC cell lines. Knockdown of IDH1 significantly decreased cell proliferation. Mechanistically, this was due to induction of senescence. We next aimed to determine the mechanism underlying senescence induction. Increased histone methylation of proliferation promoting genes (i.e., *CCNA2* and *PCNA*) is a characteristic of senescence. aKG is a cofactor for the Jumonji C histone demethylase family, suggesting that suppression of aKG may increase histone methylation. ChIP experiments indicated an increase in repressive H3K9me2 at proliferation-promoting gene loci when IDH1 was knocked down. This correlated with a decrease in mRNA expression of both genes. These data suggest that knockdown of IDH1 induces senescence of cyclin E-altered HGSC cells by increased histone methylation of proliferation-promoting genes.

Finally, we aimed to determine the Jumonji demethylase family member that is inhibited by IDH1 knockdown. KDM4A modulates H3K9, is upregulated in EOC and correlates with worse overall survival. Knockdown of KDM4A induced senescence and decreased *CCNA2* and *PCNA* expression, suggesting that KDM4A may be responsible for altered histone methylation at these loci. Altogether, these data suggest that metabolically targeting IDH1 induces senescence through epigenetic reprogramming and may be a novel metabolic therapy for cyclin E-altered HGSC patients.

P5. LonP1 differently modulates mitochondrial function and bioenergetics of primary versus metastatic colon cancer cells

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Mitochondrial Lon protease (LonP1) is a multi-function enzyme that regulates mitochondrial functions in several human malignancies, including colorectal cancer (CRC). The mechanism(s) by which LonP1 contributes to colorectal carcinogenesis is not fully understood. We found that silencing LonP1 leads to severe mitochondrial impairment and apoptosis in colon cancer cells. Here, we investigate the role of LonP1 in mitochondrial functions, metabolism, and epithelial-mesenchymal transition (EMT) in colon tumor cells and in metastasis. LonP1 was almost absent in normal mucosa, gradually increased from aberrant crypt foci to adenoma, and was most abundant in CRC. Moreover, LonP1 was preferentially upregulated in colorectal samples with mutated p53 or nuclear β-catenin, and its overexpression led to increased levels of β-catenin and decreased levels of E-cadherin, key proteins in EMT, *in vitro*. LonP1 upregulation also induced opposite changes in oxidative phosphorylation, glycolysis, and pentose pathway in SW480 primary colon tumor cells when compared to SW620 metastatic colon cancer cells. In conclusion, basal LonP1 expression is essential for normal mitochondrial function, and increased LonP1 levels in SW480 and SW620 cells induce a metabolic shift toward glycolysis, leading to EMT.

P6. Alterations of Rab7 expression affect cisplatin resistance

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Cisplatin (CDDP) is a most used chemotherapeutic drug for the treatment of different types of cancer. The majority of the platinum treated patients develop resistance to CDDP with consequent therapeutic failure. Interestingly, several reports have shown a relationship between reduced CDDP accumulation in lysosomes, defects in the lysosomal compartment and reduced cytotoxicity, or even resistance to this drug. Resistance has also been associated with increased exocytosis of lysosomal–exosomal content, including higher amounts of CDDP, and reduced size of the lysosomal compartment.

As vesicular trafficking and exosome production are regulated by Rab GTPases, we decided to investigate expression of endocytic Rab GTPases in chemosensitive and chemoresistant cells. Among RAB proteins, RAB7A is a ubiquitous member of the RAB family localized to late endosomes and lysosome. RAB7A controls transport from early to late endosomes and lysosomes in the endocytic pathway, being responsible for lysosome, phagolysosome and autolysosome biogenesis. Furthermore, RAB7A has been involved in channel trafficking, growth factor-independent survival and apoptosis. Thus, in view of the pivotal role of RAB7A in lysosome biogenesis and function, as well as channel and membrane protein trafficking, we thought of it as a good candidate to act as a regulator of CDDP-resistance.

We demonstrated that CDDP-resistant cells are characterized by a reduction of the number and size of acidic compartments and significantly downregulation of RAB7A. So, through modulation of Rab7 expression, we were able to alter CDDP response. In particular, RAB7A depletion in CDDP-sensitive cells and its overexpression in resistant counterpart determined increased resistance and sensitization to drug, respectively. ICP analysis on resistant and sensitive cells after treatment with the drug and subsequent evaluation of extracellular extracts allowed us to demonstrate that less CDDP is present inside chemoresistant cells and it correlated with increased production of extracellular vesicles. Thus, for the first time, we demonstrated that RAB7A regulates CDDP resistance determining alterations in late endocytic traffic with consequent drug efflux through extracellular vesicles. This discovery contributes to shed light on molecular mechanisms underlying chemoresistance and may lead to the design of new chemotherapeutic strategies.

P7. Targeting mitochondrial cholesterol in metabolic reprogramming of breast cancer cells

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Cholesterol metabolism is fundamentally altered in tumorigenic cells and may confer resistance to endocrine therapy in breast cancer. Trafficking of cholesterol is therefore critical to contribute to the metabolic reprogramming underlying breast neoplasia. The stress-reactive translocator protein (TSPO) which is localised to the outer mitochondrial membrane (OMM), transports cholesterol into the organelle. Notably, its expression is shown to positively correlate with tumour progression in both human and companion animal mammary tumours. We have now proven that cholesterol is involved in the mitochondrial-nuclear signalling route of cellular adaptation therefore deciding to investigate the intracellular trafficking of the lipids in both species. In order to trace cholesterol, we utilised Dehydroergosterol (Ergosta-5,7,9(11),22-tetraen-3β-ol) which is a fluorescent analogue that mimics the behaviour of native cholesterol and is readily bound by cholesterol-binding proteins and transporters. By co-staining with Ergosta (to mark cholesterol transport and accumulation) and MitoTracker® Red (to mark the mitochondrial network) we have shown that pharmacological targeting of TSPO, as well as its genetic ablation, disrupts cholesterol domains of mito-nuclear signalling in breast cancer overcoming uncontrolled proliferation. Interestingly, TSPO ligands were also able to disrupt the nuclear trafficking of ergosta in mammary epithelial cells. Furthermore, treatment with TSPO ligands prevented the nuclear accumulation of the pro-growth transcription factor, oestrogen receptor alpha (ERα) and hence aggressiveness of the lesions. These findings therefore support the importance of cholesterol in metabolic adaptation and breast cancer progression, both in domestic animals and humans, thus delineating a novel method of targeting this underlying phenomenon in breast cancer.

P8. The molecular link between Bis-Phenol A (BPA) and breast cancer

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Bis-Phenol A (BPA), used in the manufacture of clear plastic bottles and lining of food and beverage containers, has been implicated as a class 2B “suspected” carcinogen and a teratogen by several Countries. Being metabolized quickly by the liver to form DNA adducts, at low dose BPA can cause measurable DNA damage and also acts as an inhibitor of secretory pathway calcium ATPase1 (SPCA1). BPA interacts with MAPK and NFkB pathways that can lead to tumorigenesis via p53 interaction. Previous studies have either stopped at determining BPA induced DNA damage or cited the involvement of MAPK and NFkB pathways. The low and high dose exposures have differential effects on the target experimental model therefore cytotoxic or molecular studies need further investigation, especially with reference to increasing use of BPA containing products globally. Environmentally exposed populations are thus at increased risk of breast cancer induction. Our *in-vitro* experiments indicate that BPA not only causes single strand DNA breaks (SSBs) at a dose of 2ug/mL but also causes more error prone double strand breaks (DSBs) at 4 ug/mL in the BV2 murine microglial cells, MCF-7 Human breast cancer cells and normal epithelial MCF10-A cells. We have also determined that BPA mediated SPCA1 inhibition causes an upregulated expression of IGF1R surface protein, which is an early marker of breast cancer.

P9. IGF-I and hyperglycemic conditions affect BRCA1 functions as a metabolic restraint in estrogen-receptor (ER) positive breast cancer cells

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Within populations carrying the same genetic predisposition, the penetrance of *BRCA1* gene mutations has increased over time, and this has been associated with changes in lifestyle factors associated with energy metabolism. These observations are consistent with the recently described role of BRCA1 in lipogenesis. We have shown previously that in ER-positive breast cancer cell lines expressing full-length, wild-type BRCA1, IGF-I induced growth is dependent upon an increase in fatty acid synthase (FASN). BRCA1 has been shown to bind phosphorylated ACCA (Acetyl CoA Carboxylase-α), the inactive form of ACCA and this interaction reduces fatty acid synthesis. FASN is downstream of ACCA and is therefore dependent upon the balance between active and inactive forms of ACCA.

MCF7 and T47D cell lines were used as models for ER-positive breast cancer. Cells were cultured under euglyacemic and hyperglycemic conditions and treated with IGF-I (0-500ng/ml). Protein abundance was analyzed with Western blotting and cell proliferation was assessed using 3-H Thymidine incorporation assay. BRCA1 subcellular localization was determined by cell fractionation of whole cells using a NE-PER Nuclear and Cytoplasmic Extraction Reagent Kit (Thermo Scientific). The association between p-ACC (S^{79}) and BRCA1 was studied using immunoprecipitation followed by Western blotting.

We showed for the first time that the loss of BRCA1 resulted in the downregulation of a phosphorylated and inactive form of ACC, with a concomitant increase in FASN abundance. Interestingly, BRCA1 was predominantly localized in the cytoplasm of ER-positive breast cancer cells, and this localization is compatible with the observation that BRCA1 physically associates with phosphorylated ACC. Most importantly, IGF-I reduced the association between BRCA1 and p-ACC (S^{79}). Under euglycaemic conditions however, BRCA1 effectively inhibited IGF-I lipogenic actions, suggesting that BRCA1 function in lipogenesis is reduced by hyperglycemia.

Our data show that hyperglycemic conditions compromise the ability of BRCA1 to restrain IGF-I induced lipogenesis and the associated proliferative responses. Taken altogether, the evidence suggest that breast cancer risk may be reduced by making lifestyle choices that promote energy balance in the body, and this may be an opportunity for breast cancer prevention, particularly for women who carry deleterious *BRCA1* gene mutations.

P10. Pentamethinium salts are new metabolic disruptors with potential in cancer therapy

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Altered energy metabolism is a typical feature of cancer cells, which are more metabolically active than their healthy counterparts and therefore have excessive demands on energy production. Cancer cells are known for their utilization of glycolysis in aerobic conditions and their mitochondrial processes adapt for their specific needs. For this reason, energy disruptors are the subject of intensive research as potential anti-cancer drugs. In this study, we show that a pentamethinium salt derivative called TBMS-47 is potentially exploitable as a mitochondrial-targeting cancer treatment. TBMS-47 is a newly synthesized compound and we are the first to study its interesting biological properties. We have already published that TBMS-47 is selectively toxic against cancer cell lines in vitro and that it suppresses growth of transplanted tumours in mice. TBMS-47 was originally developed as a mitochondria-specific fluorescent probe and its fluorescence is a convenient feature enabling us to detect and measure it in cells. We discovered that TBMS-47 rapidly accumulates in mitochondria, followed by fragmentation of these organelles which leads to insufficient ATP production and energetic stress. Treated cells then compensate defective respiration with glycolysis and both oxygen and glucose consumption rises. Our data suggest that TBMS-47 enters cells via active transport which depends on their metabolic state. However, the precise membrane transporter is still to be discovered. Uptake of the compound can be suppressed by metabolic inhibitors as well as by specific culture conditions and nutrient availability. It is a well-established hypothesis that many cancer cells are not able to respond properly to nutrient withdrawal and TBMS-47 is a promising candidate for targeting this weak spot of cancer.

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P11. Mitochondrial levels determine variability in cell death by modulating apoptotic gene expression

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The main problem when fighting cancer is that tumor cells display fractional killing to chemotherapy. Trying to understand that resistance, we have investigated the individual cell responses to TRAIL in a clonal population of HeLa cells using live-cell microscopy and computational modelling. We found that the cellular mitochondrial content determines the apoptotic fate and modulates the time of death. Our results show that apoptotic cells have significantly higher mitochondrial content than resistant cells and highlight the amount of mitochondrial mass is a proxy for commitment to apoptosis. In addition, the correlation of mitochondrial levels with times of death indicate that cells with the highest mitochondrial levels die faster.

Cell-to-cell variability is mainly attributed to fluctuation in cellular components and metabolites, and has been implicated in the time of death in TRAIL-induced apoptosis. Since heterogeneity in mitochondrial content is responsible, through its role in energy production, for around 50% of variability in cellular protein levels, we quantified the influence of mitochondrial mass on the amounts of RNA transcripts and proteins involved in the extrinsic apoptotic pathway. Transcriptomic analysis of cells with high versus low mitochondrial content showed that apoptosis related transcripts exhibited fold-changes in expression that reflected those of the whole transcriptome. However, when we quantified the apoptotic protein content in single cells as a function of the mitochondrial mass, the data indicated that a large part of the variability observed at the protein level in the apoptotic pathway is a consequence of cell-to-cell heterogeneity in mitochondrial content.We developed a kinetic model of the apoptotic network in which the initial levels of key apoptotic proteins were introduced as a function of the mitochondrial content. Model simulations suggested that optimal cell fate discrimination by mitochondria occurs when co-variation of mitochondria with the pro-apoptotic proteins is maximal. A main hallmark of cancer cell is the Warburg effect, but also, recently, lactate has been involved as an energy source. We are now analysing the impact of that metabolic pathways on apoptotic response and resistance to chemotherapy.

P12. Pharmacological targeting of Glutamine synthetase skews macrophages toward an inflammatory phenotype and inhibits metastasis

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Glutamine synthetase (GS), the glutamine-synthesizing enzyme from glutamate, controls important events including the release of inflammatory mediators, mTOR activation and autophagy. However, its role in macrophages remains elusive. Here we report that pharmacologic inhibition of GS by Methionine Sulfoximine (MSO) skews M2-polarized macrophages toward an M1-like phenotype, characterized by a specific metabolic signature associated to HIF1α stabilization. Genetic deletion of macrophagic GS in tumor-bearing mice induces CD8+ cytotoxic T cells infiltration, blocks angiogenesis and most importantly inhibits metastasis formation. These data indicate that GS activity plays a role in the pro-metastatic function of M2-like macrophages and highlight the possibility of targeting this enzyme as a strategy to treat cancer metastasis. As a proof of concept we show that a small organic molecule able to target effectively mammalian GS at very low concentration is not toxic for mice, is able to awaken anti-tumoral functions of macrophages through a metabolic rewiring and, most importantly, to reduce the metastatic dissemination. These results might pave the way for the development of innovative immunotherapic approaches - metabo-immunotherapies- to defeat cancer.

P13. Role of mitCa^{2+} homeostasis in breast cancer

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In physiological conditions mitochondrial Ca^{2+} uptake plays a fundamental role in aerobic metabolism and ATP synthesis. On the other hand, pathological stimuli can induce mitochondrial Ca^{2+} overload which eventually leads to release of caspase cofactors and apoptotic cell death. On this basis, in a complex tumorigenic setting, fine regulation of mitochondrial calcium signaling may play a fundamental role in cell fate decision. Our laboratory investigated the role of MCU in breast cancer progression. We provided evidence that mitochondrial Ca^{2+} signaling is effectively involved in this pathology. Indeed, MCU expression correlates with tumor progression and metastasis formation, and the genetic ablation of MCU decreases breast cancer progression through a ROS/HIF1α-dependent pathway. We are now analysing the role of MCU complex regulatory subunits, MICU1 and MICU2, and of a dominant negative isoform of MCU, MCUB both in patient-derived specimens and in breast cancer cell lines. We found a significant downregulation of Micu1 in breast tumor patient's sample compared to normal breast tissues. Next, we produced stable cell lines expressing either Mcub, or Micu2, or Micus isoforms in which the Ca^{2+}-binding EF hands-domains have been mutated (Micu1^{EFmut} and Micu2^{EFmut}), thus lacking Ca^{2+}-dependent regulation. The overexpression of either Mcub or Micu2 or Micu1^{EFmut} or Micu2^{EFmut}, alone or in combination, causes a decrease in mitCa^{2+} uptake and a significant impairment in cell migration. Further studies will validate *in vitro* results in *in vivo* tumorigenesis and metastasis formation.

P14. Role of isopentenyl-diphosphate isomerase 1 in tumor angiogenesis

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Angiogenesis is the formation of new blood vessels from the pre-existing vasculature and a key component in tumor progression and metastasis development.The concept of inhibiting tumor blood vessels represents a pillar of anticancer treatment. Actually, it is known that alterations in endothelial metabolic pathways affect vessel morphogenesis and be considered an effective alternative way to anti-VEGF signalling therapies, which are often accompanied by adverse effects and tumor resistance.

Mevalonate pathway, an essential anabolic metabolic pathway that produces sterols and isoprenoid metabolites, is essential for the tumour growth. However, its role in tumor angiogenesis is still unclear. Isoprenoids are synthetized from the 5-carbon isoprene: isopentenyldiphosphate (IPP). The isomerization of unreactive IPP into its reactive isomer dimethyl-allylpyrophosphate (DMAPP) is the key rate-limiting step of the terpenoid biosynthesis and it is catalyzed by the enzyme isopentenyl-diphosphate isomerase 1 (IDI1). The aim of the present study is to investigate the role of IDI1 as possible targeting tumor angiogenesis.

We determined whether different tumor microenvironmental conditions such as hypoxia (1% O2), VEGF-A stimulation (50ng/mL), and low pH (pH=6.5) are able to regulate the expression of IDI1 in endothelial cells (HUVECs). Our results showed a significant increase at the mRNA and protein level of IDI1 at low pH, whereas no differences were observed in hypoxia and in the presence of VEGF-A. Then, we evaluated whether IDI1 knockdown is able to impair the angiogenic process.

Knockdown of IDI1 expression using a shRNA approaches blocked the proliferation and induced cell death in HUVECs, accompanied by a decrease of the protein levels of tRNAdimethylallyltransferase 1 (TRIT1). Interestingly, deferoxamine, an inhibitor of ferroptosis, rescued the lethal phenotype. In parallel, we have generated a lox allele of the IDI1 locus in mice. We are currently investigating tumor formation in VEC-CreERT2; IDIfl/fl1 mice.

Our results highlight the importance of IDI1 and isoprenoid pathway in endothelial homeostasis and provide novel evidence supporting IDI1 as a new target for tumor angiogenesis.

P15. Next generation sequencing reveals human mitochondrial DNA is heavily methylated in a cell type & cell context specific manner

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Mitochondria play critical role in cellular metabolism and control of cell death and a large body of evidence shows that cancer cells exhibit mitochondrial dysfunction and metabolic changes. Recently, alongside the discovery of mitochondrial-specific DNA methyltransferases, global and site specific methylation in the mitochondrial DNA (mtDNA) has also been described. However, high-resolution patterns of mtDNA methylation and its functional role in abnormal mitochondrial processes associated with tumorigenesis remain largely unknown and debated. Here we developed and applied a next-generation sequencing-based method for investigating mtDNA methylation to provide the first reported evidence of genome-wide human mtDNA methylation at single base pair resolution. We show that the mitochondrial genome can be extensively methylated at CpG and non-CpG sites and that baseline methylation patterns differ significantly between cell types. Importantly, these methylation patterns are also cell stage and cell context specific, with notable differences in methylation levels in tumorigenic cells compared to their primary counterparts. Furthermore, confocal imaging and silencing of DNA methyltransferase enzymes (DNMT1, DNMT3A and DNMT3B) resulted in a marked reduction in mtDNA methylation levels, indicating that these enzymes are involved in the establishment and/or maintenance of the observed baseline methylation patterns within the mitochondrial genome. Together, our results demonstrate cell context-specific methylation patterns of the mitochondrial genome that may be modulated by DNMT enzymes and suggest a role of mitochondrial DNA methylation in the disease context. These findings may open avenues for studying modes of regulation and functional consequences of aberrant mtDNA methylation in tumorigenesis and other diseases.

P16. Investigating the role of Bcl-2 family proteins in metabolism

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Mitochondria are key organelles involved in cellular respiration, ATP production, redox control and cell death. Apoptosis or programmed cell death is regulated by the Bcl-2 family proteins. These proteins are mainly located in the mitochondria and are divided into proapoptotic proteins (BH3-only, Bax, Bak…) and anti-apoptotic proteins (Bcl-2, Bcl-XL, Mcl1 ..,). We found that treatment with BH3-mimetics, drugs that bind and block anti-apoptotic Bcl-2 proteins, lead to COX-2 upregulation. COX-2 is an enzyme involved in the eicosanoid metabolism. That led us to hypothesize that BH3-mimetics may have a role in the metabolism by altering mitochondrial function. To investigate that, seahorse analysis was performed and it showed that BH3-mimetics decreased maximal respiration and mitochondrial-ATP production. Moreover, analysis of metabolites secreted to the media indicated higher glucose consumption in cells treated with BH3-mimetics, suggesting an increased glycolysis when anti-apoptotic proteins are blocked. Even more surprisingly, cells that lacked Bax and Bak, had higher respiration, indicating that the absence of those proteins could change their metabolism towards a more oxidative metabolism. Furthermore, measurement of the metabolites secreted to the media revealed that in absence of Bax and Bak, cells produce less lactate, indicating less glycolysis and confirming the change to oxidative metabolism. Moreover, analysis of the metabolites by mass spectrometry also indicated a decrease in glycolysis in the Bax and Bak knock-out cells. All those data may suggest a metabolic adaptation under BH3-mimetics treatment or in the absence of Bax and Bak that could indicate, either a direct role of Bcl-2 family proteins in the metabolism, by regulating metabolic enzymes, or an adaptive metabolic change in order to sustain cell growth. These findings may be important for the regulation and targeting of Bcl-2 family proteins in cancer.

P17. Cellular cholesterol lowering action of metformin leads to prevent proliferation and epithelial to mesenchymal transition in breast cancer cells

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Earlier literature suggetsed that metformin, a well-known anti-diabetic drug, showed anticancer activity in various cancer types. Few clinical studies showed the low serum cholesterol and low TAG level in metformin treated patients. Earlier literature also indicated an existence of positive association between high cholesterol and cancer. However, the mechanism of metformin in relation with cellular cholesterol has not yet been studied. This study aimed to find out a molecular mechanism involved in metformin-inhibited cell growth and metastasis in breast cancer cells. Tumor sample based clinical study found the higher expressions of cellular cholesterol regulatory genes (e.g. HMGCoR, LDLR) in malignant breast cancer tissues as compared to benign tissues. Our cell culture study found that treatment of breast cancer MDAMB-231 cells with metformin decreased cellular cholesterol level with concomitant inhibition of various genes (e.g. SREBF1 and LDLR) which maintain the homeostasis of cellular cholesterol. Cell cultue based experimental study documented that Metformin inhibited cell proliferation, migration, colony and spheroid formation of metatstatic breast cancer MDA-MB231 cells. As a mechanism it was identified by RT-PCR that metformin treatment inhibited antiapoptotic markers (Bcl2, BCLxl) and mesenchymal marker genes (Vimentin, N-cadherin) transcript levels with simultaneous enhancement of apoptotic markers (Caspase3, Bax) and epithelial marker genes E-cadherin and Keratin19, indicated an inhibitory effect of Metformin in proliferation and EMT of breast cancer cells. Less number of colony and spheroid formation has been observed in Metformin treated breast cancer cells. RT-PCR analysis also found that Metformin treatment inhibited stemness marker CD44 and BMI-1 in metastatic breast cancer MDA-MB-231 cells. Moreover, Metformin-inhibited cancer cell proliferation and migration was reversed by the exogenous treatment of cholesterol. Similarly, cholesterol treatment reversed the metformin-inhibited Bcl2, Vimentin, BMI-1 expression. Moreover, zymography data documented that cholesterol treatment upregulated metformin-inihibited MMP activity. These findings unravel a significant contribution of cellular cholesterol on EMT and stemness of breast cancer cells since cholesterol depleting MBCD inhibited cancer cell proloferation and EMT. Moreover, high level of cholesterol and cholesterol regulatory gene expressions was noted in cancer tissues. This study suggests a new molecular mechanism where metformin inhibits proliferation, EMT and stemness of breast cancer cells presumably by lowering cellular cholesterol level.

Keywords: Breast Cancer, Epithelial to mesenchymal transition, Stemness, Metformin, Cholesterol

P18. Nrf2 is required for initiation and progression of lung cancer in Kras mutant mouse model

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Lung cancer develops through cumulative acquisition of mutations and epigenetic changes associated with constitutive activation of different cell growth pathways. These include genes encoding KRAS, EGFR, MET and PIK3CA, or loss of the tumor suppressors like p53 and PTEN. The cancer genome atlas (TCGA) consortium identified that the master regulator of oxidative stress response pathway, nuclear factor-erythroid 2 p45-related factor 2 (NRF2 encoded by NFE2L2) is dysregulated in 25-35% of lung cancers through mutations in its repressor Kelchlike ECH-associated protein-1 (KEAP1). Somatic mutations in KEAP1 were reported as second most common mutation in lung ADC (adenocarcinoma) and fourth most common in lung SQCC (squamous cell carcinoma) by TCGA. Also, gain-of-function mutations in NFE2L2 were reported to be the sixth most common in SQCC. Besides mutations in KEAP1 and NFE2L2, NRF2 is also up-regulated in KRAS mutant tumors.

We aimed to determine the significance of Nrf2 upregulation in lung carcinogenesis using a Nrf2 'dose-dependent' strategy in an established Kras driven mouse model of lung cancer. To investigate the effect of varied levels of Nrf2 activity in lung tumorigenesis, we crossed the KrasLSL-G12D/+ mice with Keap1fl/fl, Keap1+/fl ,Nrf2-/- and Nrf2+/- mice to generate following five mice lines in order of their increasing Nrf2 activity: Nrf2-/::Keap1+/+::KrasLSLG12D/+;Nrf2+/::Keap1+/+::KrasLSLG12D/+;Nrf2+/+::Keap1+/+::KrasLSL-G12D/+; Nrf2+/+::Keap1+/fl::KrasLSLG12D/+ and Nrf2+/+::Keap1fl/fl::KrasLSL-G12D/+. The Nrf2 expression in the five lines correlated with the expression levels of antioxidant-, glutathione-, thioredoxin- and NADPH generating- related genes. Histopathological examination of Nrf2-null mice showed no or minimal development of lung lesions 12- and 24-weeks post K-ras oncogene activation. On the other hand, mice expressing varied levels of Nrf2 developed lung lesions. Further, increase in Nrf2 activity led to development of more aggressive, high-grade tumors in these mice. We found that the redox status (GSH/GSSG) in lungs of these mice correlated to their Nrf2 expression levels. Significantly, diminished levels of GSH in Nrf2-null mice appears to have resulted in failure of oncogenic K-ras to cause lung tumour development in these mice. Therefore, Nrf2 is required for the initiation and progression of lung cancer.

P19. Dynein light chain induced oligomerisation of Bim and Bmf regulates mitochondrial apoptosis by determining their stability and neutralisation by anti-apoptotic Bcl-2 proteins

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Mitochondria are the major organelle involved in cellular energetics and apoptosis. Evasion of apoptosis is a hallmark of cancer initiation and progression. During initiation of apoptosis, mitochondrial outer membrane permeabilization (MOMP) by the Bcl-2 family proteins leads to the release of apoptogenic proteins and loss of mitochondrial membrane potential. These events activate caspases as well as compromise the metabolic functions of mitochondria causing cell death. Therefore MOMP is often considered a point of no return for mitochondrial apoptosis. BH3-only members of the Bcl2-family activate the mitochondrial pore-forming proteins Bax/Bak. Dynein light chain (DLC) has been proposed to negatively regulate the pro-apoptotic activity of BH3- only proteins Bim and Bmf. However, the underlying molecular mechanisms still remain elusive. We found that DLC induces the formation of homo-oligomeric complexes of Bim or Bmf on mitochondria. This complex formation was an important factor in determining the stability of Bim and Bmf as well as their interaction with anti-apoptotic Bcl-2 proteins. Loss of DLC also leads to the degradation of Mcl-1, a pro-survival Bcl-2 protein contributing to resistance against different anticancerous agents. Intriguingly, we also found that DLC mediated hetero-oligomerization of Bmf and Bim in a cell-free system and in intact cells. This suggested that Bim and Bmf might compete for binding to DLC. Interestingly, over-expression of wild-type Bmf but not BmfAA (DLC binding mutant) caused substantial loss of endogenous BimEL in MEFs and in HoxB8 neutrophil progenitor cells (NPC); supporting our earlier finding that DLC regulates Bim stability. Surprisingly, the levels of Mcl-1 in these cells remained unchanged indicating that Bmf might stabilize Mcl-1 in the absence of Bim. Bmf overexpression sensitized the HoxB8 NPCs to apoptotic stimuli. However, no significant difference in the pro-apoptotic activity of Bmf and BmfAA was noticed despite considerable differences in their endogenous Bim levels. This study provides a new mechanism for the regulation of BH3-only proteins Bim and Bmf by DLC.

P20. Defining the metabolic role of glutamine synthetase in β-catenin-driven hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the most frequent liver cancer and the third most common cancer related-death worldwide. The WNT/β-catenin pathway is frequently hyperactive in HCC ultimately leading to the stabilization and increased transcriptional activity of β-catenin. Among the genetic alterations that activate the WNT signaling pathway in liver, mutations in the β-catenin gene are the most frequent, and they are found in ~25% of HCC patients. Glutamine is the most abundant amino acid in blood and a crucial source of nitrogen for cells. Glutamine can be synthetized by glutamine synthetase (GS), using glutamate, ammonia and ATP. Thus, high expression of GS in tumors has been associated with the glutamine-independent growth. β-catenin regulates glutamine metabolism, on one hand, by increasing the expression of GS (glutamine synthesis), on the other hand by increasing glutaminase (i.e. glutamine catabolism) via cMYC. Interestingly, the co-occurrence of hyperactive β-catenin and c-MYC is found in HCC patients, and is sufficient to cause HCC in mice. Whereas in normal liver the expression of GS is confined to a specific area surrounding the central vein, mutations in β-catenin lead to an ubiquitous expression of GS in hepatocytes. However, the metabolic role of GS overexpression and its relevance in tumor initiation and progression has not been elucidated in β-catenin driven HCC. Using crisp/cas9 we generated GS KO cells from β-catenin-mutant HCC cell lines (HepG2 and HuH6). In both cell line, the GS deletion affects the proliferation upon glutamine deprivation. Of note, in the Huh-6 cells the genetic depletion, as well as the pharmacological inhibition of GS, decreases cell proliferation even in the presence of physiological levels of glutamine in the medium. To address the role of GS in HCC biology we generated a genetic mouse model driven by β-catenin/c-MYC in which GS will be deleted conditionally in the liver. These models will shed light on the metabolic hallmarks of a genetically define subtype of HCC, and will allow us to test if and how glutamine synthesis plays a role in HCC tumorigenesis.

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