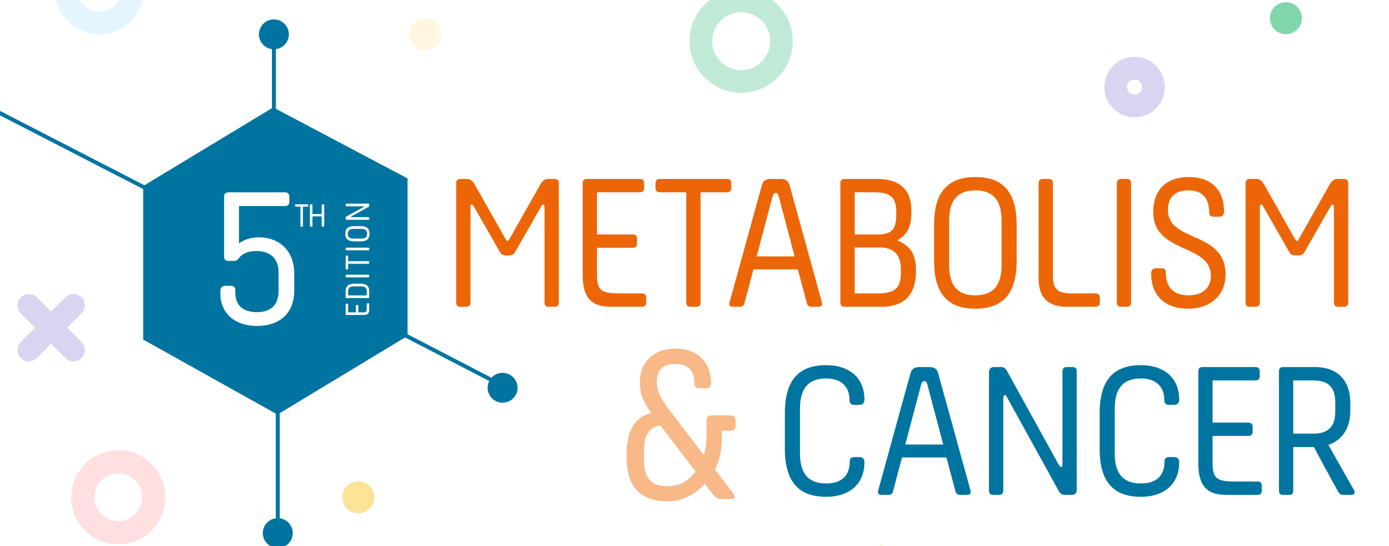
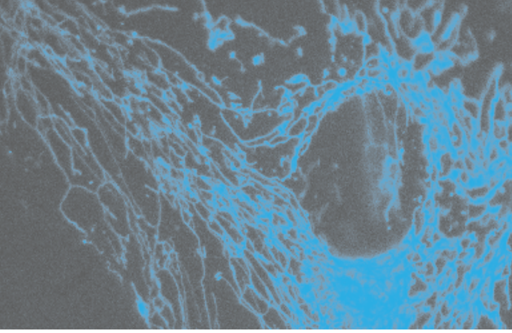


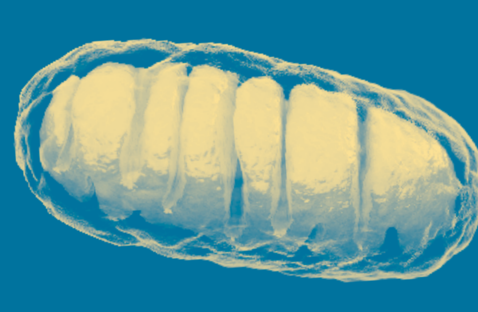
METABOLISM & CANCER

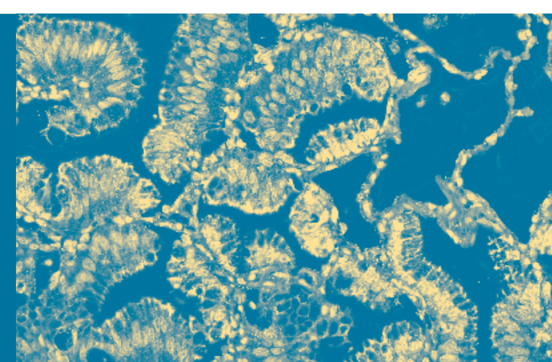
Nice, FRANCE

BOOKLET OF PARTICIPANTS

NOVEMBER 22nd-24th 2023







SCIENTIFIC COMMITTEE

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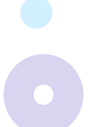
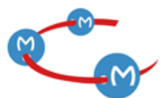




METABOLISM-CANCER.COM

WELCOME TO NICE!

It is with a great pleasure that we welcome you to the 5th edition of the Metabolism & Cancer symposium in the picturesque city of Nice. Nine years have passed since the inaugural edition here and following successful events in Palavas les flots (2016), Marseille (2019) and Bordeaux (2021), the meeting is becoming one of the premier event for fans of Cell Metabolism in oncology in France and Europe. One of the signature of the meeting is to give the opportunity to young researchers to present their work and share their original unpublished results.

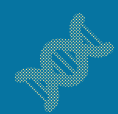
Nice, the capital of the French Riviera, is renowned for its stunning beaches and sunny weather. The city has been a source of inspiration for famous artists like Henri Matisse, Raoul Dufy and Marc Chagall. The Old Town of Nice, located not far from our meeting venue at the magnificent St Paul Hotel, stands as one of the city's main attractions. Beyond its historical significance, it offers a vibrant atmosphere day and night – an ideal to explore, relax with a drink or savor a traditional "Niçois" meal.

This edition features an outstanding scientific program, including internationally renowned speakers, with a focus on Immunometabolism, metabolism in tumor microenvironement, ferroptosis and drug resistance as well as therapeutic strategies targeting metabolism. The Saint Paul Hotel provides a splendid backdrop for three days of insightful discussions.

We hope this symposium provides a platform for inspiring discussions with colleagues, fostering potential collaborations for the future. Our sincere thanks go to all regional institutions, national associations "La Ligue Nationale Contre le Cancer", "Canceropôle Sud", "Société Française du Cancer", as well as our private sponsors who have played a crucial role in organizing of this significant event. Thanks all of those who have dedicated their time and energy to ensure the success of this meeting.

We look forward to welcoming you in Nice and hope this symposium proves to be an inspiring and stimulating experience for all participants.

The organizing committee



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MAIN TOPICS

- IMMUNOMETABOLISM
- DRUG RESISTANCE, FERROPTOSIS & METABOLISM
- THERAPY AND METABOLISM
- MICROENVIRONMENT

12.45 PARTICIPANTS WELCOME

13.30 OPENING & INTRODUCTION
Frédéric Bost, C3M, Nice, France

13.40 17.15 **SESSION 1**
IMMUNOMETABOLISM
Chairmen: Jean-Emmanuel Sarry & Jean-Ehrland Ricci

13.40 **KEYNOTE LECTURE 1**
Reprogramming metabolic crosstalk in tumors for anti-cancer treatment
Ping Chih Ho, Lausanne, Switzerland

14.10 **SHORT COMMUNICATION 1**
Improving T cell activation by limiting mitochondrial-derived ATP transfer to the cytosol
Omri Yosef, Jerusalem, Israel

14.25 **Mechanisms of resistance to amino acid targeting in B-cell lymphomas**
Johanna Chiche, Nice, France

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14.45	**KEYNOTE LECTURE 2** **Harnessing tumor metabolism to overcome immunosuppression** *Massimiliano Mazzone, Leuven, Belgium*
15.15	**SHORT COMMUNICATION 2** **Unraveling the Impact of Muscle Heme Metabolism on Tumor Microenvironment and Pre-metastatic Niche** *Alessio Menga, Turin, Italy*
15.30	**Dissecting the role of arginine pathway in melanoma aggressiveness and therapy resistance** *Michael Cerezo, Nice, France*
15.50	**Coffee break**
16.20	**SHORT COMMUNICATION 3** **Plasticity of one-carbon metabolism and its exploitation for toxic folate trapping in cancer cells** *Johannes Meiser, Strassen, Luxembourg*
16.35	*Maxim N. Artyomov, Saint Louis, USA*
16.55	**Importance of metabolism in mast cell regulation: from allergy to leukemia.** *Fabienne Brenet, Marseille, France*
17.15	**SESSION** **Bioenergetic of Hypoxic Glycolysis controls tumors, pathogens, immunity and tissue repair - Genetic deconstruction and therapeutic perspectives** *Chairmen: Nathalie Mazure & Laurent Le Cam* *Special Guest : Jacques Pouysségur, Nice, France*
17.45 19.30	POSTERS SESSION 1 & COCKTAIL

November 23RD 2023

9.00 – 12.15 SESSION 2
DRUG RESISTANCE / FERROPTOSIS / METABOLISM

Chairmen: Alice Carrier & Laurent Le Cam

9.00 KEYNOTE LECTURE 3
Unexpected Guardians: 5,7-Unsaturated Sterols as Cytoprotective Metabolites
Pedro Friedmann Angeli, Würzburg, Germany

9.30 An Atlas of Ferroptosis-induced Secretomes
Silvia Von Karstedtt, Cologne, Germany

9.50 SHORT COMMUNICATION 4
Cancer-associated fibroblast-induced lipid metabolic reprogramming promotes drug resistance in ALK-driven lung adenocarcinomas
Ann-Kathrin Daum, Heidelberg, Germany

10.05 Host and tumor metabolism driving drug resistance in acute myeloid leukemia
Jean-Emmanuel Sarry, Toulouse, France

10.35 Coffee break

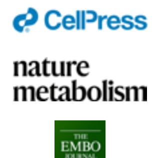
11.05 Metabolic stress in the tumor microenvironment
Cristina Muños-Pinedo, Barcelona, Spain

11.25 Deciphering the metabolic determinants of GBM cell adaptation to stress
Erika Cosset, Lyon, France

11.45 SHORT COMMUNICATION 5
Overcoming resistance to venetoclax in AML: a radical approach
Vittoria Raimondi, Padova, Italy

12.15 Editors short presentation

Luca Gasparoli, CELLPRESS
Alfredo Giménez-Cassina, Nature Metabolism
Daniel Klimmeck, EMBO Journal



12.30 Lunch

13.15 Round Table with the Editors

November 23RD 2023

14.30
17.15

SESSION 3
THERAPY, TARGET AND METABOLISM

Chairmen: Stéphane Rocchi & Rodrigue Rossignol

14.30 **KEYNOTE LECTURE 4**
The multiple faces of two-carbon metabolism in cancer progression and therapeutic
Eyal Gottlieb, Houston, USA

15.00 **SHORT COMMUNICATION 6**
Targeting Metabolic Dependencies of Translation in Neuroblastoma
Raphael Morscher, Zürich, Switzerland

15.15 **Glutamine addiction in the CLL microenvironment; towards therapeutic applications and a PET tracer as a novel diagnostic tool**
Eric Eldering, Amsterdamn, The Netherlands

15.35 **Coffee break**

16.10 **Non-canonical micropeptides in rewiring mitochondria function of head and neck cancer**
Anna Sablina, Leuven, Belgium

16.40 **SHORT COMMUNICATION 7**
A metabolic synthetic lethality revealed by PI3K addiction in leukemia
Guillaume Andrieu, Paris, France

16.55 **Unexpected Activation of the Bile Acid pathway! Cholesterol a key metabolite in ccRCC tumorigenesis**
Romain Riscal, Montpellier, France

17.15
19.30

POSTERS SESSION 2 & COCKTAIL

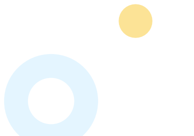
17.20 **INDUSTRY TALKS**

17.20 **Explore Agilent immuno-oncology research tools for assessing real-time cell function, phenotype, and fate**
Damien Brechet

17.25 **High-throughput phenotyping for your cells**
Gaëtan Podeur

17.30 **Precision medicine enabled by quantitative metabolomics**
Carlos Malpica

20.00 CONGRESS DINNER - HÔTEL ASTON



November 24TH 2023

9.00
12.00

SESSION 4
MICROENVIRONMENT METASTASIS

Chairwomen: Nathalie Mazure & Sophie Vasseur

9.00 **KEYNOTE LECTURE 5**
Metabolic rewiring driving metastasis formation
Sarah-Maria Fendt, Leuven, Belgium

9.30 **SHORT COMMUNICATION 8**
Mechano-dependent sorbitol accumulation supports biomolecular condensate
Stéphanie Torrino, Nice, France

9.45 **SHORT COMMUNICATION 9**
A metabolic crosstalk occurs between human bone marrow adipocytes and prostate cancer cells
Marine Hernandez, Toulouse

10.00 **The BMP signaling differently affects stem cell functions depending on their resident tissue: from in breast early transformation to bone marrow metastatic cells dormancy**
Véronique Maguer-Satta, Lyon, France

10.30 **Coffee break**

11.00 **Migration, mechanosensing and metabolism in pancreatic ductal adenocarcinoma invasion and metastasis**
Laura Machesky, Glasgow, UK

11.20 **How glioblastoma cells adapt their metabolism when colonizing the brain ?**
Thomas Daubon, Bordeaux, France

11.40 **KEYNOTE LECTURE 6**
Metabolic Alterations in Tumor and Host in Cancer
Eileen White, New Brunswick, USA

ABSTRACTS

Improving T cell activation by limiting mitochondrial-derived ATP transfer to the cytosol

Omri Yosef [1] *, Michael Berger [1],

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Introduction: CD8+ T cells' metabolic programs are critical for their function and fate decisions. As the metabolic hub of the cell, the mitochondria play a crucial role in T cell-mediated immunity. However, the mechanisms by which mitochondria govern T cell responses and cellular fate are not completely understood. Notably, inhibition of ATP-synthase using oligomycin impaired activation, yet decoupling respiration from ATP synthesis using FCCP restored T cell proliferation by reinvigorating the TCA cycle. This observation proposed an augmented OXPHOS in activated T cells fuels not only ATP synthesis but also anabolic TCA activity. By delving into the significance of ADP/ATP translocase 2 (Ant2), a key player in mitochondrial ATP transport, through both inhibitory and genetic approaches, we disclosed that Ant2 deficiency surprisingly did not impede T cell activation or proliferation, despite its anticipated adverse effects on OXPHOS and the TCA cycle. However, the metabolic adaptations naïve Ant2-/- T-cells undergo and their impact on development, activation, and function remain unclear.

Methods: To unveil the underlying mechanisms adapted by CD8+ T cells to function independently from mitochondrial ATP, we tested how reducing mitochondrial ATP from the CD8+ T cells' cytoplasmic compartment affect their activation by generating a T cell-specific Ant2-/- model. Ant2 deficiency leads to a reduction in mtATP to the cytosol, accompanied by a concomitant decrease in ADP concentrations within the mitochondrial matrix. Consequently, the functionality of the ATP-synthase complex is hindered, resulting in a mitochondrial membrane hyperpolarization. This sustained perturbation negatively affects the efficiency of the electron transport chain, thereby inducing oxidative stress through the disruption of OXPHOS and the subsequent impairment of NAD+ regenerative capacity. The cascade of events collectively contributes to the altered bioenergetic landscape in Ant2-deficient T cells.

Results: Remarkably, we found that Ant2 deficiency improved CD8+ T cell activation, proliferation and effector functions. Following these findings, we demonstrated that naïve Ant2-/- CD8+ T cells overcome restrained OXPHOS capacity by increasing their mitochondrial biomass, spare respiratory capacity, and through the induction of selective anabolic pathways. Specifically, these pathways include aerobic glycolysis, TCA-cycle activity, ROS production, aspartate biosynthesis & nucleotides biosynthesis, and proline biosynthesis. The changes observed in naive Ant2-deficient T cells echo the metabolic rewiring characteristic of T-cell activation, albeit driven by a distinct rationale.

Conclusion: Overall, our study provides a better understanding of the metabolic programs adapted by CD8+ T cells to support rapid proliferation and effector functions. the insufficiency of OXPHOS in activated cells mirrors the OXPHOS inhibition arising from Ant2 absence. We propose that OXPHOS inhibition and its ensuing cascade of events in Ant2-deficient naive T cells prompt an adaptive response akin to the metabolic programming witnessed in primed cells. As a substantial portion of their metabolic rewiring has already occurred, these naive T cells appear to bypass the typical metabolic reprogramming associated with priming. Consequently, these unique naive T cells manifest activated-like metabolic characteristics and behaviors.

Wednesday November 22ND 2023 - 15:20
SESSION 1 - IMMUNOMETABOLISM

Unraveling the Impact of Muscle Heme Metabolism on Tumor Microenvironment and Pre-metastatic Niche.

Alessio Menga [1] *, Myriam Hsu [2], Alfonso Scalera [1], Roberta Basile [1], Rita Vacca [1], Ivan Zaggia [1], Laura Conti [1], Emanuela Tolosano [1], Paolo Porporato [1],

1. Molecular biotechnology and health sciences, University of torino, Torino, ITALY
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Background
Metastasis accounts for at least 90% of cancer-related fatalities worldwide. Although existing research has primarily concentrated on intrinsic alterations within cancer cells that facilitate their dissemination throughout the body, the potential influence of metabolic alterations in the host on the metastatic process remains largely uncharted. Extensive metabolic shifts in cancer result in systemic inflammation and muscle mass depletion. We postulated that muscle wasting might affect tumor progression and the spread of metastases by supplying metabolic resources. Our investigation using Lewis Lung Carcinoma (LLC) tumor-bearing mice unveiled reduced levels of iron and heme in skeletal muscle, primarily attributable to heightened transcription of the cytoplasmic heme exporter FLVCR1a in muscles affected by tumors.

Methods
To clarify the relevance of muscle-derived heme in tumor progression and metastatization, we generated a skeletal-muscle specific knock-out mouse model for the heme exporter (FLVCR1a fl/fl ; MyoD Cre+/- C/57). Subsequently, we introduced Lewis Lung Carcinoma (LLC) cells subcutaneously to assess both tumor growth and metastatic potential. Tumor growth was monitored by measuring with calipers twice a week over a span of 28 days. To evaluate the extent and number of metastases, we utilized H&E staining of lung tissue samples. We conducted FACS analysis to determine the percentage and phenotypic characteristics of immune cells that had infiltrated the primary tumors. Additionally, we employed the Proteome Profiler Murine Cytokine Array Kit to identify cytokines associated with metastasis in the serum. Our investigation also encompassed RT-qPCR and Western Blot analysis to examine variations in the expression of genes related to inflammation and heme/iron metabolism in skeletal muscle, tumors, and other organs. To gain further insights into metabolic changes and iron levels, we employed RNAseq analysis for skeletal muscle and ICP-MS analysis for bone marrow.

Results
Although there was no discernible difference in tumor growth, the silencing of FLVCR1a in skeletal muscle resulted in a significant reduction in pulmonary metastatic nodules. This implies that alterations in heme metabolism within muscle tissue could exert an influence on the tumor microenvironment, thereby facilitating the intravasation and invasion of cancer cells. In mice with tumors, where muscular FLVCR1a was present (FLVCR1a WT) in comparison to those where it was silenced (KO), heme acted as a Damage-Associated Molecular Pattern (DAMP), triggering the activation of the Toll-Like-Receptor 4 (TLR4) pathway within skeletal muscle. This activation, in turn, led to the production and release of Myonectin and pro-metastatic cytokines, such as TIMP-1. Myonectin, a stress-induced myokine belonging to the TNF-α related protein family, played a role in inhibiting hepatic hepcidin, thus promoting the mobilization of iron from the liver and increasing the availability of iron for the production and maturation of neutrophils in the bone marrow. Ultimately, mature neutrophils infiltrated the primary tumors, enhancing vessel permeability through the activity of myeloperoxidase (MPO), which in turn supported the intravasation and spread of cancer cells.

Conclusions
In summary, our findings suggest that the expression and/or function of FLVCR1a in muscle tissue regulate local inflammation and likely impact systemic immunity, ultimately promoting the progression of cancer to metastasis.

Keywords : Lung Metastasis, Skeletal Heme Metabolism, Neutrophils, Metabolic Crosstalk, Intravasation

Wednesday November 22ND 2023 - 16:00
SESSION 1 - IMMUNOMETABOLISM

Plasticity of one-carbon metabolism and its exploitation for toxic folate trapping in cancer cells

Johannes Meiser [1],

1. Department of cancer research, Luxembourg institute of health, Luxembourg, LUXEMBOURG

One-carbon metabolism is predominantly known for its essential function to support nucleotide synthesis in proliferating cancer cells. However, during recent years several laboratories including ours have provided profound evidence that one-carbon metabolism can support various cellular functions and that the output of one-carbon metabolism can be tailored towards current cellular needs. This plasticity of one-carbon metabolism actively supports metastasis formation. In this context, we have demonstrated that cancer cells excrete a significant amount of one carbon units in form of formate (formate overflow). Increased extracellular formate concentrations prime cancer cells towards a pro-invasive phenotype. Interestingly, inhibition of formate overflow strongly reduces the metastatic potential of cancer cells without limiting their proliferative capacities, indicating growth-independent functions of one-carbon metabolism. While we have proven a causal relation between formate overflow and metastasis, approaches to exploit formate overflow for targeted cancer cell killing remained elusive until recently. Using a novel small molecule targeting MTHFD, we discovered a novel folate trap that is installed in formate overflow positive cancer cells. Intriguingly, physiological concentrations of hypoxanthine enhance the trapping effect resulting in cancer cell death due to thymidylate depletion. This novel mechanism is of particular interest as MTHFD2 belongs to the most upregulated enzymes in various tumour types and is instrumental to promote formate overflow.

Keywords : formate, metastasis, serine, one-carbon metabolism

Cancer-associated fibroblast-induced lipid metabolic reprogramming promotes drug resistance in ALK-driven lung adenocarcinomas

Ann-Kathrin Daum [1] *, Lisa Schlicker [2], Marc A. Schneider [3], Thomas Muley [3], Ursula Klingmüller [4], Almut Schulze [5], Petros Christopoulos [6], Michael Thomas [6], Holger Sültmann [1],

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Introduction: Targeted therapy interventions using tyrosine kinase inhibitors (TKIs) provide compelling response rates in anaplastic lymphoma kinase-positive (ALK+) lung adenocarcinoma patients (1). The subsequent emergence of treatment resistance, however, still poses a major clinical challenge (2). Several lines of evidence support a crucial role of cancer-associated fibroblasts (CAFs) to such treatment insensitivities, while underlying mechanisms remain poorly understood (3). In this context, we aimed to uncover molecular networks shaping the therapeutic susceptibility of lung cancer cells towards ALK inhibition via tumor stromal cues in 3D co-culture settings.
Methods: 3D-culture conditions were applied to ALK+ lung cancer cell lines and CAFs to enhance the pathophysiological relevance of in vitro modeling. Flow cytometry-based functional analyses were performed to assess drug responses of ALK+ lung tumor spheroids in a CAF-dependent manner. Stroma-driven alterations of biological processes and underlying molecular mechanisms in ALK+ lung adenocarcinoma cells as a consequence to ALK inhibition were investigated utilizing single-cell transcriptional profiling. Transcriptional differences were interrogated between homo- and heterotypic lung tumor spheroids following ALK signaling perturbations. Potential routes of heterocellular communication between tumor cells and CAFs were examined through ligand-receptor-interaction analysis. The expression and functional relevance of in silico predicted CAF-secreted factors were validated by immuno- and cell viability assays. MS-based metabolome analysis was conducted to determine differences in the metabolic profile of TKI-treated and co-cultured lung tumor spheroids.
Results: Here, we show that CAFs were able to provoke resistance to the ALK-TKIs brigatinib and lorlatinib in H2228 and H3122 lung tumor spheroids as observed by reduced cell death rates and increased cell cycle progression. Transcriptional differences between homo- and heterotypic lung tumor spheroids as interrogated by differential gene expression analysis of scRNA-sequencing data revealed a CAF-driven increase of lipogenic targets (e.g. FASN, SCD1, SREBF1) upon ALK inhibition, a finding which was further validated in vitro. Ligand-receptor-interaction analysis predicted heterocellular communication between CAFs and tumor cells via HGF and NRG1, whose abundance was additionally confirmed in CAF-conditioned medium (CM). CAF-CM and CAF-associated ligands HGF and NRG1β1 were likewise able to mediate therapy resistance, which was concomitant with a re-expression of lipogenic proteins and AKT signaling induction. Furthermore, interference with ALK signaling inhibited de novo lipogenesis, while increasing lipid peroxidation. This effect was attenuated by CAF-mediated paracrine signaling. Importantly, the established CAF-driven lipid metabolic-supportive niche of TKI-resistant lung tumor spheroids could be overcome by simultaneous targeting of ALK and SREBP-1, a prominent regulator of lipogenesis.
Conclusion: By employing a systematic approach that integrated advanced 3D-cell cultures of ALK+ lung cancer cell lines and CAFs along with scRNA-sequencing, we gained insights into distinct cell (sub)population-specific gene expression variabilities in response to external stimuli. Our findings additionally unveiled a novel role played by CAFs in promoting resistance to ALK inhibition through alterations in cancer lipid metabolism.
Together, these discoveries emphasize the importance of CAF-driven non-genetic resistance and provide avenues for potential treatment strategies to address TKI resistance by targeting vulnerabilities downstream of oncogenic signaling. Such strategies could hold promise for enhancing the clinical outcomes of ALK+ lung adenocarcinoma patients.

Keywords : lung adenocarcinoma, cancer-associated fibroblasts, therapy resistance, lipid metabolism, 3D-cell culture

Overcoming resistance to venetoclax in AML : A radical approach

Vittoria Raimondi [1] *, Francesco Ciccarese [2], Endri Mauro [3], Fabrizio Carta [4], Andrea Angeli [4], Giulia Ciotti [5], Alessandra Sperotto [5], Julie Mondet [6], Claudiu Supuran [4], Michele Gottardi [5], Pascal Mossuz [6], Vincenzo Ciminale [1],

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Introduction. Most AML patients exhibit resistance to the current therapies and are thus incurable. Disease relapse is common and often fatal. The Bcl-2 inhibitor venetoclax was approved by the FDA for the treatment of AML in combination with hypomethylating agents and improves the outcomes of older patients or those unfit for intensive chemotherapy. However, AML cells frequently become resistant to venetoclax, and new strategies to overcome this resistance are urgently needed. This study was aimed at overcoming venetoclax resistance by rewiring redox homeostasis in leukemia cells.

Materials and Methods. Building on pilot experiments with DHEA, an inhibitor of glucose 6-phosphate dehydrogenase (G6PD), which leads to inhibition of NADPH production and increase of ROS levels, we develop a panel of new G6PD inhibitors. The affinity of these compounds for the target protein was evaluated through an in silico molecular docking analysis. The response to venetoclax alone or in combination with the most promising drugs was tested in a panel of 6 AML cell lines and 35 primary samples from patients. Cell death and mitochondrial ROS accumulation were evaluated by flow cytometry, and NADPH levels were measured using a luminometric assay. The synergistic effect on cell death of our drugs and venetoclax was assessed using the Bliss method. Long-term culture experiments were also performed to test the efficacy of our treatments over time. The specificity of these compounds for leukemic cells was verified by analyzing their effects on healthy cells. Finally, we investigated the association between response to venetoclax and metabolic alterations by evaluating their content in mitochondria, lipids, and amino acids through HRMAS-NMS and GC-MS. Mitochondrial function was evaluated through a Seahorse flux analysis.

Results. Both AML cell lines and primary samples from patients exhibited different degrees of resistance to venetoclax. However, the combination between venetoclax and inhibitors of the G6PD significantly increased the sensitivity to venetoclax, with a mean 50-fold reduction in the 50% cytotoxic concentration (CC50). Notably, Bliss analysis revealed that the interaction between these compounds was highly synergistic. Importantly, the combination of venetoclax with G6PD inhibitors proved to be very effective in eliminating cancer cells in long-term cultures of primary patient samples. Moreover, the G6PD inhibitors did not increase the cytotoxic effect of venetoclax in normal cells, suggesting that this approach could dramatically improve its clinical efficacy and possibly reduce side effects by allowing a reduction of its dosage. Interestingly, we observed that total lipid abundance, amino acid content, and oxygen consumption correlate with the different responses of AML cells to venetoclax and G6PD inhibitors. These observations provide the rationale to target these metabolic hubs to decipher the mechanism of action of our combined treatments and kill refractory AML cells.

Conclusions. Our results indicate that manipulation of redox homeostasis is a promising strategy to enhance the efficacy of venetoclax in AML and could increase its therapeutic window.

Keywords : AML, ROS, venetoclax, G6PD

Thursday November 23RD 2023 - 15:00
SESSION 3 - THERAPY, TARGET AND METABOLISM

Targeting Metabolic Dependencies of Translation in Neuroblastoma.

Raphael J. Morscher [1] *, Sarah Cherkaoui [1], Lifeng Yang [2], Matthew Mcbride [2], Turn Christina S. [3], Wenyun Lu [2], Caroline Eigenmann [1], George E. Allen [4], Olesya O. Panasenko [4], Lu Zhang [5], Annette Vu [3], Om H. Gandhi [3], Lea Surrey [6], Michael Wierer [7], Eileen White [5], Joshua D. Rabinowitz [2], Michael D. Hogarty [3],

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Cancer growth is fueled by nutrients obtained from circulation and local biosynthesis. Great progress has been made to understand cancer's dependence on nutrient uptake using in vivo stable isotope tracing. Exploiting these specific dependencies via dietary manipulation to potentiate anti-cancer therapies is undergoing active research. Here we show that simultaneous targeting of translation by defined amino acid depletion, combined with the clinically approved drug difluoromethylornithine (DFMO), induces neuroblastoma tumor growth inhibition and differentiation. We characterize the arginine-proline-glutamine axis as a source of polyamine precursors and identify that both patient tumors and in vivo neuroblastoma models harbor high proline levels in MYCN-amplified disease. In vivo stable isotope tracing identifies uptake from circulation as the primary tumor source of proline and ornithine, the direct polyamine precursor. On an organismal level, arginine feeds tumor ornithine via circulation. Depletion of proline and arginine from the diet limits circulatory substrate availability and enhances intratumoral polyamine depletion by DFMO. As a result, we find disruption of translation by deficient posttranslational modification of the elongation initiation factor 5 A (eIF5A) and most prominently, a new likely eIF5A unrelated phenotype. Multi-omics integration, including high-resolution ribosome profiling and proteomics, reveals polyamine depletion-induced translation defects by increasing ribosome stalling at adenine-ending codons. Reprogrammed translation induces cell cycle arrest and neuronal differentiation causing significant and durable anti-tumor response in the TH-MYCN transgenic model of neuroblastoma. Thus, we provide a proof-of-concept for employing combined targeting of metabolic dependencies of translation to inhibit the growth of cancers dependent on external nutrient uptake.

Keywords : Protein translation, polyamines, difluoromethylornithine, amino acid dependency, diet, proline, arginine, glutamine

Thursday November 23RD 2023 - 16:40
SESSION 3 - THERAPY, TARGET AND METABOLISM

A metabolic synthetic lethality revealed by PI3K addiction in leukemia

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Introduction
T-cell acute lymphoblastic leukemia (T-ALL/T-LL) is an aggressive cancer of children and young adults, marked by poor clinical response, notably for refractory and relapsing (R/R) cases. As in numerous cancers, genetic lesions leading to aberrant PI3K signaling (PI3KSALT) are frequent in T-ALL and convey adverse outcomes, urging innovative therapies for these patients. We explored the metabolic plasticity of PI3K-driven leukemia to unravel targets amenable to novel strategies for this aggressive disease.

Methods/Patients
Patients were enrolled in the GRAALL2003-2005 and FRALLE2000 trials. Patient primary samples were analyzed by pan-exome sequencing, arrayCGH, MLPA and RNA sequencing. Patient-derived xenografts (PDX) were used for ex vivo and in vivo experimentation. PI3K signaling activity, glucose consumption and cell survival were evaluated by flow cytometry. Metabolomics analyses were carried out at our Metabolomics Core. The treated patients were enrolled in the Registry of Relapsed/Refractory T-cell Acute Lymphoblastic Leukemia (NCT05832125).

Results
PI3KSALT patients had a poor response to corticoids, a shorter overall survival (5y-OS: 58% vs 74%, p = 0.007) and event-free survival (5y-EFS: 49% vs 65%, p = 0.008), and an increased incidence of relapse (5y-CIR: 39% vs 27%, p = 0.01). PI3KSALT define a subclass of aggressive T-ALL with poor prognosis.

Primary samples and PDX of PI3KSALT leukemia have an hyperglycolytic profile. PI3KSALT T-ALL cell lines mimic this addiction to glucose. Surprisingly, while cell lines cannot survive glucose limitation, PI3KSALT PDX tolerate this starvation, underpinning their ability to rewire their metabolism to adapt to a nutrient-deprived microenvironment. We showed that PI3KSALT PDX have unique metabolic plasticity upon starvation. These blasts use glutaminolysis to cope with glucose limitation and sustain the TCA cycle, while wild-type PI3KS fail to do so (Fig1A). Pharmaceutical inhibition of the PI3KS-mTOR pathway and glutamine metabolism presented a marked cytotoxicity ex vivo (Fig1B).

We proposed a therapeutic strategy for PI3KSALT T-ALL based on targeting mTOR and glutamine. Erwinase, an L-asparaginase with glutaminase activity, efficiently synergizes with Torisel and demonstrates tumor clearance and prolonged survival in vivo (Fig1CD). Critically, we report five patients suffering from R/R PI3KSALT leukemia, with complex clinical history, treated with the association Erwinase/Torisel (ET). All five patients achieved a rapid response. Patients suffering from R/R T-ALL achieved negative minimal residual disease. Patients with R/R T-LL presented a significant decrease in the mediastinal mass (Fig1E). After complete remission, three patients received consoli-

dation therapy consisting of allogeneic SCT or donor lymphocyte infusion. Two patients remain alive, while two patients rapidly progressed after the discontinuation of ET treatment.

Conclusion
R/R T-ALL/LL have dismal prognosis and outcomes, in part due to chemoresistance acquisition and limited therapeutic options. We show that metabolic plasticity conveys a unique and targetable vulnerability in PI3KS-driven leukemia. We propose a promising treatment combining an asparagine and glutamine degrader (Erwinase) with a PI3KS inhibitor (Torisel) that should be considered as a therapeutic option in a bridge-to-transplant approach for R/R T-ALL/LL with PI3KS deregulation. The Registry of Relapsed/Refractory T-cell Acute Lymphoblastic Leukemia (NCT05832125) is open to evaluating the efficacy of Erwinase-Torisel in RR-T-ALL/LL.

Keywords : T-cell acute lymphoblastic leukemia, PI3K, metabolic plasticity, glutamine, targeted therapy

Friday November 24TH 2023 - 09:30
SESSION 4 - MICROENVIRONMENT METASTASIS

Mechano-dependent sorbitol accumulation supports biomolecular condensate

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Biomolecular condensates regulate a wide range of cellular functions from signaling to RNA metabolism, yet, the physiologic conditions regulating their formation remain largely unexplored. Biomolecular condensate assembly is tightly regulated by the intracellular environment. Changes in the chemical or physical conditions inside cells can stimulate or inhibit condensate formation. However, whether and how the external environment of cells can also regulate biomolecular condensation remain poorly understood. Increasing our understanding of these mechanisms is paramount as failure to control condensate formation and dynamics can lead to many diseases. Here, we provide evidence that matrix stiffening promotes biomolecular condensation in vivo. We demonstrate that the extracellular matrix links mechanical cues with the control of glucose metabolism to sorbitol. In turn, sorbitol acts as a natural crowding agent to promote biomolecular condensation. Using in silico simulations and in vitro assays, we establish that variations in the physiological range of sorbitol, but not glucose, concentrations, are sufficient to regulate biomolecular condensates. Accordingly, pharmacologic and genetic manipulation of intracellular sorbitol concentration modulates biomolecular condensates in breast cancer – a mechano-dependent disease. We propose that sorbitol is a mechanosensitive metabolite enabling protein condensation to control mechano-regulated cellular functions. Altogether, we uncover molecular driving forces underlying protein phase transition and provide critical insights to understand the biological function and dysfunction of protein phase separation.

Keywords : Biomolecular condensate; Mechanobiology; Cell metabolism; Glucose metabolism; Sorbitol

Friday November 24TH 2023 - 09:50
SESSION 4 - MICROENVIRONMENT METASTASIS

A metabolic crosstalk occurs between human bone marrow adipocytes and prostate cancer cells

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In localized prostate cancer (PCa), we have demonstrated that periprostatic adipocytes increase tumor progression by providing cancer cells with fatty acids (FAs) released after the activation of lipolysis, involving the hydrolysis of triglycerides (TG) [1]. In advanced PCa, the majority of metastases are found within the bone, where tumor cells can interact with bone marrow adipocytes (BMAds). However, whether a metabolic crosstalk between primary BMAds and PCa exists and favors tumor progression remains to be determined. Thanks to a collaboration with orthopedic surgeons, we obtain human bone marrow adipose tissue (BMAT) during hip replacement surgery. There are two types of BM-Ads: those contained in the red BMAT (rBMAds) and those contained in the yellow BMAT (yBMAds), which have been characterized by my team [2]. Since PCa metastatic sites are frequently found in proximity to rBMAds, we established a 3D culture of these adipocytes in a fibrin matrix to preserve their viability for up to 5 days and cultured them with PCa cells.
Under coculture conditions, PCa cells exhibited an increase in neutral lipid content, primarily composed of TG. Using rBMAds loaded with

fluorescent FAs, we directly demonstrated that FAs released by rBMAds are taken up by cancer cells and re-esterified into TG. These data provide the first evidence of a metabolic crosstalk between primary human rBMAds and PCa cells. Through lipidomic approaches, we determined that rBMAds release FAs mainly palmitate, oleate, and linoleate. However, like yBMAds [2], we found that rBMAds are devoid of lipolysis due to a profound decrease in the expression of the last two enzymes of the lipolytic pathway. These data suggest that an original mechanism, independent of classical lipolysis, may be involved in the release of FAs by rBMAds. Interestingly, the first lipolytic enzyme, ATGL (Adipose Triglyceride Lipase), and its cofactor are expressed in rBMAds and could participate in the release of FAs through an unusual incomplete lipolytic process which is currently under investigation. Once inside tumor cells, we found that FAs are stored as TG but are also oxidized in mitochondria. However, this increased fatty acid oxidation is not associated with increased ATP production. Thus, FAs taken up by PCa cells are not primarily used for energy production but could be involved in other processes, such as transcriptomic remodeling. RNASeq and gene ontology analyses of PCa cells cocultivated with or without rBMAds reveal clear differences in migration pathways. This process is a key step in the propagation of cancer cells from one bone metastatic site to other bone metastatic site, making the disease highly aggressive. Functional experiments confirmed that rBMAds specifically increase the migratory capacity of different PCa cell lines without any increase in proliferation. Whether this pro-migratory effect of rBMAds is due to the transfer of FAs is under investigation.
In conclusion, the metabolic crosstalk between rBMAds and PCa cells could contribute to the propagation of bone metastasis. Deciphering this crosstalk, including other metabolites, could lead to pharmacological targets for the treatment of bone metastases, for which therapeutic options remain very limited.

Keywords : human bone marrow adipocytes, prostate cancer, lipids, metabolic crosstalk, tumor progression

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P1 - Variation in lipid species profiles among leukemic cells significantly impacts their sensitivity to the drug targeting of lipid metabolism and the prognosis of AML patients

Caroline Lo Presti 1, Yoshiki Yamaryo-Botte 2,

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Several studies have linked bad prognoses of acute myeloid leukemia (AML) to the ability of leukemic cells to reprogram their metabolism and, in particular, their lipid metabolism. In this context, we performed “in-depth” characterization of fatty acids (FA) and lipid species in leukemic cell lines and in plasma from AML patients.

The leukemic cell lines were cultured under basal conditions, in serum-free medium, and with Etomoxir, which is a fatty acid oxidation (FAO) inhibitor. The cells were analyzed by gas chromatography coupled with mass spectrometry (GC-MS). The plasmas of 39 patients diagnosed with de novo AML (excluding AML3) were collected and analyzed by GC-MS.

We firstly showed that leukemic cell lines harbored significant differences in their lipid profiles at steady state, and that under nutrient stress, they developed common mechanisms of protection that led to variation in the same lipid species. This highlights that the remodeling of lipid species is a major and shared mechanism of adaptation to stress in leukemic cells.
We also showed that sensitivity to Etomoxir was dependent on the initial lipid profile of cell lines, suggesting that only a particular “lipid phenotype” is sensitive to the drug targeting of FAO.
We then showed that the lipid profiles of plasma samples from AML patients were significantly correlated with the prognosis of the patients. In particular, we highlighted the impact of phosphocholine and phosphatidylcholine metabolism on patients’ survival.

In conclusion, our data show that balance between lipid species is a phenotypic marker of the diversity of leukemic cells, that significantly influences their proliferation and resistance to stress, and thereby, the prognosis of AML patients.

Keywords : acute myeloid leukemia; metabolism; lipid species; inhibition of FAO

P2 - Variation in lipid profiles of leukemic cells: a major determinant of the prognosis of AML patients

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Keywords : acute myeloid leukemia, metabolism, lipid species

P3 - Crosstalk between EZH2, lipid metabolism and stemness in paediatric diffuse midline glioma

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Introduction: Diffuse Midline Glioma H3K27-altered (DMG) is a rare and dismal brainstem tumour affecting children with a 5-year overall survival below 2%. Chemotherapy has not shown any improvement and radiotherapy remains the gold standard in DMG treatment but is not curative. These tumours are characterised by an upregulation of EZH2, a multifunctional protein whose canonical function is to trimethylate H3K27 through the Polycomb Repressive Complex 2 (PRC2). The use of EZH2 inhibitors in the treatment of DMG is still matter of debate. In 2022, our team showed the benefit of combining EZH2 inhibitor GSK126 with cholesterol-lowering statins to eradicate DMG cells. However, the mechanism of this synergistic effect is unclear. Methods: To decipher this process, we used various molecular and cellular approaches, including flow cytometry, gene and protein expression measurements and a live-cell imaging system in DMG cells treated with GSK126 or grown in different culture media. Results: We first confirmed that GSK126 induces the neo-synthesis of lipids and the expression of lipid metabolism-related genes in DMG cell lines and further showed an up-regulation of stem cell markers in GSK126-treated cells. Conversely, EZH2 silencing with siRNAs reduced the expression of all these genes and markers. Furthermore, we generated isogenic gliomaspheres and differentiated glioma cells, which exhibited a similar response to GSK126 or siRNA treatment, respectively. Similarly to differentiated DMG cells after GSK126 treatment, gliomaspheres were more sensitive to statins than differentiated cells. Moreover, GSK126 inhibited the repressive histone mark H3K27me3 and favoured the active histone mark H3K27ac. A similar pattern was observed with gliomaspheres compared to differentiated DMG cells demonstrating a close relationship between EZH2 functions, histone marks, lipid metabolism and cancer stemness. Conclusion: Here, our data support the idea that GSK126 eliminates most differentiated DMG cells and reprograms a subset of cells which express lipids and stemness features. Further investigations on the reciprocal importance of stemness induction and lipid synthesis are needed to provide a clearer understanding of the synergistic effect of EZH2 inhibitors and statins.

Keywords : DMG, lipid metabolism, stemness, drug, EZH2

P4 - Pro-/anti-proliferative effects of hexanoic acid treatment on tumor cells of different origins

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Introduction

The metabolic reprogramming known as the “Warburg effect” is one of the most reliable hallmarks of cancer cells; it consists of the transition from oxidative phosphorylation to glycolysis, even in the presence of adequate oxygen concentrations. The process efficiently responds to the need of the cancer cell to have faster energy availability to cope with increased cell proliferation1 and at the same time it could represent a therapeutic approach. Indeed, the stimulation of oxidative metabolism, is a highly studied approach in order to inhibit the “Warburg effect” and cancer cell proliferation.

Among the different approaches used to induce oxidative metabolism we stimulate the lipolysis of intracellular neutral lipids2. The released

fatty acids were oxidized into mitochondria leading to increased oxidative metabolism2. The side effect of boosted mitochondria is the higher production of reactive oxygen species (ROS), whose buffering depends on the functionality of cellular antioxidant systems3. Here, we report the data of cell response to the treatment with a medium-chain fatty acid, which can directly be oxidized at the mitochondrial level.

Materials and methods
We exploited a short-chain fatty acid, namely hexanoic acid, to study the effects of increased lipid catabolism on aggressiveness of HeLa cells, derived from cervical cancer, and HepG2 cells, derived from hepatocarcinoma. Techniques of cell counting (e.g. Trypan Blue, Crystal Violet) were used to evaluate hexanoic acid consequences on tumor cells proliferation. In parallel, the role of main antioxidant enzymes was analyzed by following the expression levels and by assessing their activity. Moreover, the glutathione antioxidant system was considered by determining its levels by HPLC.

Results
The proliferative response of the two cell lines to hexanoic acid treatment was completely opposite; while HepG2 cells showed an arrest in the proliferative rate, HeLa cells showed an increase. This result was paralleled by a different ROS production, the levels of which were unchanged in HepG2 cells but significantly increased in HeLa cells. Consistently, the expression of the main antioxidant enzymes was increased in HepG2 cells and decreased in HeLa cells. We demonstrated that increased proliferation in HeLa cells was strictly dependent on increased GSH levels. Indeed, by treating cells with BSO, a known inhibitor of GSH synthesis, we observed an increase in cell death by the ferroptotic pathway.

Conclusion
In this study, we provide evidence for the crucial role of ROS in the metabolic response of cancer cells to fatty acids-induced mitochondrial metabolism. We highlighted that the antioxidant adaptation of cancer cells can be predictive of the proliferative effects of lipid catabolism laying the ground for the use of ROS modulation as a fruitful therapeutic approach.

Keywords : Antioxidants, Lipid catabolism, Mitochondrial metabolism, ROS, Ferroptosis

P5 - Characterization of WM9 and Hs294T malignant melanoma cell lines resistant to BRAF/MEK inhibitors

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Introduction
Malignant melanoma is one of the most dangerous and invasive types of cancer. One of the main reasons for its high aggressiveness is occurrence of BRAFV600E mutation, present in approximately 50% of patients 1. Currently used form of therapy is based on BRAF and MEK inhibitors, however, it often leads to the development of resistance 2. There are several mechanisms accompanying this phenomenon such as genetic alternations, MAPK pathway reactivation, metabolic reprogramming, changes in the cell's interactions with the tumor microenvironment, the presence of cancer stem cells (CSCs) and occurrence of epithelial–mesenchymal transition (EMT) 3. The aim of this study was to characterize obtained resistant melanoma cell lines, which may help deepen our knowledge about drug resistance.

Methods
Resistant melanoma cell lines were obtained using increasing concentrations of vemurafenib and cobimetinib (BRAF and MEK kinases inhibitors, respectively). A Cell Proliferation Kit II was used to measure proliferation rate and cell viability. To measure protein level Western Blot analysis was performed. To explore the expression level of selected genes qRT-PCR analysis was conducted. Cytokine Array Kit was used to determine cells' secretome.

Results
Two human melanoma cell lines resistant to BRAF and MEK kinase inhibitors were developed. Obtained cells are less sensitive to the BRAF/MEK inhibitors in comparison to the control. Moreover, lower sensitivity to inhibition of phosphorylated ERK1/2 kinase, which is one of the effectors of BRAF and MEK proteins, was observed in resistant melanoma cells. These cells exhibited also a decrease in the proliferation rate compared to the control as well as a decrease in the expression of selected cyclins. We also noticed an increase in activation of signaling pathways and elevated expression of the MET, EGFR and ERBB2 receptors in resistant cells in comparison to control. Moreover, transport protein ABCA1 level was elevated in resistant melanoma cell lines. We also observed increased expression of markers of CSCs as EMT process. Finally, we observed increased level of selected cytokines and interleukins in the secretome of resistant cells in comparison to control cells.

Conclusions
Our results have shown a number of changes that occur after the emergence of resistance in human melanoma cells. We believe that characterization of resistant melanoma cell lines will allow us to expand our knowledge about the phenomenon of drug resistance and in the future it might contribute to the development of new, more effective therapies for patients with malignant melanoma in its late stage of development.

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2 Halloush, S. et al. Ann. Pharmacother. 57, (2023)
3 Manzano, J. L. et al. Ann. Transl. Med. 4, 1–9 (2016)

This research was funded by the National Science Centre, Poland, with a grant received by Aleksandra Simiczyjew (SONATA 16, 2020/39/D/NZ5/02330).

P6 - Chemoresistance in breast cancer: The role of ABCB1 and NOTCH3

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Taxanes, in particular paclitaxel (PTX) and docetaxel, are widely used in the treatment of breast cancer. However, repeated administration can result in the development of chemoresistance, leading to therapy failure. Several mechanisms of resistance to taxanes have been described, one of them being the drug efflux mediated by ATP-binding cassette (ABC) transporters, particularly ABCB1 (P-glycoprotein). To circumvent the resistance to taxanes, modified taxoids called Stony Brook Taxanes (SB-Ts) have been developed. Recently, in the context of chemoresistance, NOTCH3 has received attention, as evidence indicates that unresponsive breast cancer patients have altered expression of the NOTCH3 signaling pathway. Therefore, to further elucidate the role of ABCB1 and NOTCH3 in the resistance to taxanes, we generated ABCB1 knock-out (KO) and overexpressing (OE) and NOTCH3 KO, normal and PTX-resistant breast cancer cells. By studying the cell proliferation and death, we confirmed that SB-Ts are effective even in cells with high expression of ABCB1. Furthermore, we detected a cross-resistance to mitochondrially targeted iron chelators (mitoDFO, mitoDFX), a new class of chemotherapeutic compounds recently introduced by our group, in the PTX-resistant and ABCB1 OE cells. Interestingly, paclitaxel-resistant cells have high mRNA and protein levels of Bcl-2 contrary to ABCB1 OE cells, suggesting further adaptations that make them less susceptible to cell death induction. Moreover, our results indicate that NOTCH3 KO cells are less sensitive to taxanes and mitochondrially targeted iron chelators. Mechanistically, we observed that while mitoDFO enhances extracellular acidification rate (ECAR) in normal cells, it does not affect ECAR in the NOTCH3 KO cells, suggesting that the effect of mitoDFO on energetic metabolism is different in these cells. In addition, NOTCH3 likely affects estrogen signaling, given that NOTCH3 KO cells have greater amounts of estrogen receptor alpha than normal cells based on western blot. Collectively, our results indicate that SB-Ts can serve as potential alternatives to conventional taxanes in chemoresistant cancer cells and point to NOTCH3 signaling pathway as a potentially relevant player in the chemoresistance in breast cancer.

Keywords : chemoresistance, taxanes, ABCB1, NOTCH3, mitochondrially targeted iron chelators

P7 - Cholesterol Enrichment: A Protective Mechanism Against Lysosomotropic Drugs

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Numerous small molecule drugs and inhibitors tend to be trapped in lysosomes, disrupting lysosomal functions and reducing drug efficacy. These drugs, known as lysosomotropic agents, are typically characterized by their basic nature (pKa > 6) and amphiphilic properties (logP > 2). They are transported to the lysosome and subsequently protonated through a pH partitioning process, rendering them membrane impermeable and causing them to be trapped within the lysosome. Altering the pH of lysosomes by sequestering weak bases impairs lysosomal functions, hightlighting necessity of understanding the consequences of these modifications.

Several chemotherapy drugs targeting various types of cancer exhibit lysosomotropic properties. Notable examples include tamoxifen, imatinib, gefitinib, lapatinib, and sunitinib. Through RNA sequencing, we identify cholesterol synthesis as one of the most significantly activated pathways following treatment with lysosomotropic drugs, particularly in resistant cells. Our qPCR analysis demonstrates that the expression of genes involved in cholesterol synthesis increases up to 10-fold when the lysosomal pathway is interfered by lysosomotropic drugs. Furthermore, with LC-MS-based lipidomic analysis, we observe a more than 2-fold increase in cellular cholesterol levels upon lysosomotropic treatment. Notably, blocking cholesterol synthesis with statins sensitizes cells to lysosomotropic treatment, resulting in reduced cell viability. Our findings suggest that the enrichment of cholesterol within cells plays a protective role against lysosomal stress. Future research will focus on gaining a deeper understanding on the role of cholesterol in drug resistance and the benefits it provides during lysosomotropic drug treatments.

Keywords : chemotherapy, lysosomotropism, cholesterol, drug resistance

P8 - Blocking the poliovirus receptor energizes natural killer cells to overcome cetuximab resistance in head and neck squamous cell carcinoma.

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Antibody dependent cellular cytotoxicity by NK cells can overcome cetuximab resistance. However in the TME NK cells are suppressed whereby anti-tumor activity is limited. We identified the poliovirus receptor to be crucial and highly expressed in HNSCC patients. Blocking the PVR synergistically enhances the degree of ADCC and helps to overcome cetuximab resistance.

Keywords : Cetuximab, ADCC, NK cells

P9 - Characterization of the antitumor properties of phenylamino-1,3,5-triazine (PAT) a new family of ferroptosis inducers.

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Growing evidence suggests that cancer cells resistant to chemo-, targeted or immunotherapies remain highly sensitive to ferroptosis, a programmed cell death playing a paramount role in tumor control. Here, we performed a medium throughput phenotypic screen on new compounds synthesized at the Nice Chemistry Institute to identify a promising new family called phenylamino-1,3,5-triazine (PAT).
PATs decrease the viability of cell lines from a wide range of tumor type in the low micromolar range, without significant toxicity for normal cells. Moreover, they demonstrate a high efficacy in vivo on mice models of xenograft. A transcriptomic analysis of PAT-treated cells revealed a ferroptosis and NRF2 signature. Subcellular fractionation confirmed the nuclear translocation and DNA binding of NRF2 resulting in the expression of antioxidant proteins. In line with this observation, PAT treatment induces a rapid accumulation of Fe^{2+}, ROS and ultimately lipid peroxidation that constitute the hallmarks of the ferroptotic process. Thus, the effects of PATs are almost entirely blunted by ferroptosis inhibitors.
Mechanistically, PATs inhibit cell respiration resulting in an induction of the AMPK pathway that appears to be mandatory for the induction of ferroptosis. Consistent with the fact that therapy-resistant cancer cells switch to a more OXPHOS metabolism, we observed an increased efficacy of PATs on melanoma cells resistant to targeted therapies.
In conclusion, the high efficacy targeting of therapy-resistant cancer cells by PATs provides compelling data to pursue its evaluation as a potential drug for cancer patients in therapeutic failure.

Keywords : Ferroptosis inducer, melanoma, small molecules, therapy resistance

P10 - Respiratory CI inhibitors in anti-cancer therapy - NDUFS3 knockout cancer cells and molecular docking as tools for evaluating specificity and mode of action

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INTRODUCTION: Inhibition of respiratory complex I (CI) as anti-cancer strategy has shown promising results in preclinical studies (1). However, despite the advances in the development of new CI inhibitors, none of them is currently used in clinical practice and several open issues remain.

MATERIALS and METHODS: We here evaluated the antiproliferative potential and mode of action of five CI inhibitors, with the aim of identifying the most efficient and least toxic compound in the context of cancer treatment. In particular, metformin, EVP 4593, BAY 87-2243, IACS-010759 and a novel quinone analogue (QA) were tested in cell lines representative of aggressive ovarian cancer, colon cancer and melanoma, as well as in patient-derived non neoplastic cells. In vitro and in vivo antiproliferative assays have been performed, as well as the inhibitor specificity control using unique CI knock-out models and molecular docking prediction to compare the mode of action (2).

RESULTS: All tested drugs, apart from metformin, have shown to selectively target CI at concentrations efficient to induce antiproliferative effect in cancer models, which was mainly cytostatic rather than apoptotic. A preliminary in vivo study revealed that the novel QA is efficient in reducing tumor progression, equivalently to the already known CI inhibitors.

CONCLUSION: Our data make a case for caution when referring to metformin as a CI-targeting anticancer compound, and highlight the need for dosage optimization and careful evaluation of molecular interactions between inhibitors and the holoenzyme.

P11 – Investigating osimertinib resistance in lung cancer using untargeted lipidomics and cell biology

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Introduction
Non-small-cell lung cancer (NSCLC) accounts for 80% of lung cancer cases. Epidermal growth factor receptor mutations (EGFRm) occur in approximately 15% and 40% of NSCLC in Western and Asian populations, respectively. Current treatment targets EGFRm with tyrosine-kinase inhibitors (TKIs). Resistance to these TKIs however is inevitable. Osimertinib is a 3rd generation EGFR-TKI now used as a first line treatment in the advanced/metastatic setting, exhibiting an average progression free survival of 18.9 months. Dysregulation of metabolism has been suggested to play a role in development of drug resistance. Here, we investigate how aberrant lipid metabolism contributes to the development of osimertinib-resistance (OR) using pharmacologically and genetically-induced resistance models.

Materials and Methods
Pharmacologically-induced resistant cell lines were generated by chronically dosing PC9 cells with increasing concentrations of osimertinib to a maximum of 160nM, producing PC9R5 and PC9R6 resistant clones. Genetically-modified resistant lines were generated through CRISPR gene editing producing a PC9 PTEN-knockout line and a PC9 line harbouring a PIK3CA H1047R mutation. Resistance phenotype was determined using a cytotoxicity assay. Next generation RNA sequencing (RNAseq) data was generated using the Affymetrix Expression console and collected on 4 resistant clones, including PC9R5, to investigate OR. Pathway enrichment was performed using Gene Set Enrichment Analysis. Untargeted lipidomics was carried out on pharmacologically and genetically-modified OR lines and their sensitive controls. Lipids were extracted using a modified Folch method and analysed using liquid chromatography-mass spectrometry (LC-MS/MS) on an LTQ-Orbitrap Elite.

Results
We probed the RNAseq data for differentially expressed genes pertaining to key lipid metabolic pathways and found significant changes in genes involved in ceramide metabolism, such as upregulation of UGCG which encodes glucosylceramide synthase (GCS). Lipid profiles of OR compared to osimertinib-sensitive (OS) cells were distinct in both resistance models. In the pharmacological model, we detected a relative decrease in ceramides and increase in glycosphingolipids (e.g. hexosyl ceramides) in OR versus OS cells. We additionally detected relative increase in polyunsaturated fatty acid (PUFA)-containing phospholipids and triacylglycerols (TGs) in OR versus OS cells. Plasmalogens were found to be decreased in PC9R5 versus OS but increased in PC9R6. In the genetic models, a relative decrease in PUFA-containing phospholipids and TGs was observed in PC9 PIK3CA-mutation line relative to OS, however this was not observed in the PC9 PTEN-knockout line, which did not have a significantly different lipid profile to OS.

Conclusions
The lipidomic profile of OR and OS cells are distinct in the pharmacological model of resistance, with changes to ceramide metabolism noted. Ceramides promote apoptosis via caspase-3 activation, therefore it is advantageous for tumour cells to convert ceramide to glucosylceramides via GCS. Similarly, by reducing the pool of free ceramide in favour of glucosylceramides, OR cells may be less susceptible to apoptosis. These lipid changes were not detected in genetic models of resistance, suggesting that other mechanisms play a role in these mutants. Further work will include treating pharmacologically-induced OR cells with C2-ceramide and inhibiting GCS to determine if sensitivity to osimertinib can be reinstated by increasing the pool of free ceramide.

Keywords : NSCLC, EGFR-TKI, osimertinib, lipidomics, drug resistance, metabolism, ceramides

P12 - Characterization of tumor metabolic reprogramming to overcome acquired therapeutic resistance in colorectal cancer

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Introduction
Colorectal cancer (CRC) is the third most common cancer worldwide and a leading cause of cancer-related mortality. Genes that have a role in cell cycle control are frequently altered in cancer. Cyclin-dependent kinases CDK4 and CDK6 (CDK4/6) are promising targets for inhibiting cell cycle progression since their overexpression is implicated in a wide range of human cancers. Like with all targeted therapies, tumor cells eventually acquire resistance to CDK4/6 inhibition. Despite the great therapeutic advances, chemotherapy resistance remains one of the biggest challenges in its treatment. Accumulating evidence suggests that, under an environmental pressure such as a chemotherapeutic treatment, cancer cells undergo cell-reprogramming processes to optimize survival, therefore becoming resistant. We hypothesize that tumor metabolic adaptations in response to antineoplastic drugs represent de novo vulnerabilities that can be targeted to overcome acquired drug resistance. In this context, an outstanding challenge is to develop new tools to understand and target relevant metabolic mechanisms underlying the acquisition of drug resistance in CRC to forestall therapeutic failure.

Materials and methods
We used metabolomics and transcriptomics techniques to analyze the metabolic reprogramming associated with CDK4/6 inhibition in colon tumor-derived cells. Then, we constructed and applied Genome-Scale Metabolic Models (GSMMs) to unveil new targets able to prevent the metabolic reprogramming sustaining drug resistance in CRC.

Results
In the present study, we have used GSMMs and the quadratic metabolic transformation algorithm (qMTA) to characterize the metabolic adaptation underlying CRC therapeutic resistance to CDK4/6 inhibitors (such as palbociclib, abemaciclib, and ribociclib) with demonstrated anticancer therapeutic efficacy. We observed that the absence of functional CDK4 and CDK6 causes a metabolic reprogramming that includes enhancing amino acid metabolism, oxidative phosphorylation, and oxygen consumption. Identifying the metabolic changes associated with therapy resistance facilitates the rational design of new therapies that combine established chemotherapy treatment options with new drugs targeting these metabolic key players. The resistance-hindering targets predicted by this new strategy can be experimentally validated to define new effective combination therapies.

Conclusion
The adaptive responses of cancer cells after chemotherapeutic treatment emerge as metabolic dependences and vulnerabilities that can be targeted to overcome acquired drug tolerance and resistance, resulting in strong synergistic antiproliferative effects.

Keywords : Metabolic reprogramming, Therapeutic resistance, Cell cycle, GSMM, Colorectal cancer

P13 - Chemotherapy-resistant TP53-mutated acute myeloid leukemia cells have an altered transcriptional state and metabolic profile that drive venetoclax resistance

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Introduction: Despite good responses in the initial treatment phase, the biggest challenge in treatment of acute myeloid leukemia (AML) is persistence of residual therapy-resistant cancer cells (minimal residual disease, MRD) that can develop into recurrence. Especially TP53-mutated AML patients have an extremely poor treatment outcome compared to non-TP53-mutated AML patients after chemotherapy- or venetoclax-based treatments. Current knowledge gaps on ways to target MRD, especially in TP53-mutated AML, are hampering progress in development of therapies successfully preventing relapse and improving AML cure rates.
Aim: Identification of characteristics and vulnerabilities of chemotherapy- and venetoclax-resistant (TP53-mutated) AML, guiding the design of treatment strategies successfully eliminating AML relapse-initiating cells.

Methods&Results: To study intra-leukemia heterogeneity with respect to anthracycline sensitivity, we cultured the stem cell-like TP53-mutated AML cell line KG1, 10.000 cells/well in 350 wells, with increasing concentrations of daunorubicin (up to 23.2ng/ml). After six weeks of treatment, five wells showed survival of cells, suggesting clonal outgrowth of therapy-resistant AML cells. These daunorubicin-resistant clones (DaR-clones) could be cultured over a long period of time in presence of 23.2ng/ml daunorubicin, with similar growth rates to wild-type (WT) cells. The DaR-clones showed 5.6-6.9 times lower sensitivity to daunorubicin than WT cells. Interestingly, all DaR-clones were also resistant to the BCL2 inhibitor venetoclax, to concentrations up to 12.5µM, suggesting that the major mechanisms driving daunorubicin resistance in these clones are also driving venetoclax resistance. Targeted next generation sequencing of WT cells, using VariantPlex myeloid panel, revealed presence of a major TP53 c.672+1G>A mutation (variant allele frequency (VAF) 90%), known to be present in KG1, and two minor TP53 mutations (c.721T>G, VAF 5%; c.705C>G, VAF 5%). All DaR-clones were derived from cells containing the major TP53 c.672+1G>A mutation (VAF>98%), and two clones gained a novel mutation with low VAF (a GATA2 mutation (VAF 5%) and SETBP1 mutation (VAF 7%), respectively).
As it was demonstrated that venetoclax resistance in AML cells include dependencies on alternative BCL2 family members and reprogramming of their metabolism, we investigated whether daunorubicin treatment could affect expression of anti-apoptotic BCL2 family members and pro-apoptotic proteins BAX and BIM (together calculated as apoptotic index (AAI)). Using western blot and flow cytometry no clear differences in BCL2, MCL1, BCL-xL, BAX and BIM expression and AAI were found in the DaR-clones compared to WT cells.
To identify mechanisms of dual chemotherapy-venetoclax resistance we performed gene expression profiling, using RNA-sequencing. This revealed hydroxycarboxylic acid receptor 1 (HCAR1) and BCL2 family apoptosis regulator BOK (BCL2L9) as top genes upregulated in the DaR-clones compared to WT+dauno and WT cells. Pathway analysis of the top differentially expressed genes in the DaR-clones compared to WT cells revealed modulated metabolic pathways, glycerolipid metabolism, and amino-acid metabolism, suggesting that daunorubicin-resistant AML cells have altered metabolic profiles that could drive resistance to venetoclax.

Conclusion: We showed that chemotherapy-resistant AML cells display altered metabolic profiles, potentially resulting in venetoclax resistance. This metabolic adaptability of AML cells might guide the design of treatment strategies eliminating AML relapse-initiating cells.

Keywords : acute myeloid leukemia, dual chemotherapy-venetoclax resistance, metabolic adaptibility

P14 - CD36: a double-edged sword in breast cancer progression?

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Introduction

Breast cancer is the leading cancer in terms of incidence and mortality in women. Breast tumours develop in a complex microenvironment whose main component is adipose tissue. The degree of invasion of the adipose tissue by tumour cells reflects their aggressiveness. We previously shown that mammary adipocytes promote the aggressiveness of breast tumour cells through the overexpression of the long-chain fatty acid transporter CD36 [1]. CD36 has been shown to play a role in metabolism as well as in tumour aggressiveness in many cancers. Furthermore, recent studies have pointed out the role of CD36 in an emerging regulated cell death called ferroptosis which is caused by the accumulation of lipid-based reactive oxygen species (ROS) [2,3]. Our hypothesis is that CD36 is a potentially signature of metastasis-initiating cells but at the same time could sensibilize those cells to ferroptosis.

Materials and methods
Stably overexpressing CD36 clones from two breast cancer cell lines with different molecular subtypes and aggressiveness (MCF7; luminal A and SUM159; triple negative) were generated and treated with saturated (palmitate) and unsaturated (oleate) fatty acids in two-dimension (2D) and three-dimension (3D). The viability, the lipid storage capacities, the aggressiveness, their sensibility to ferroptosis and their metabolism were analyzed according to CD36 protein expression. Cell death was evaluated by flow cytometry using Annexin-V and ferroptosis was evaluated by Bodipy-C11 staining as well as by the study of key ferroptotic gene expression by RT-qPCR.

Results
First, viability tests showed a higher sensitivity of the most aggressive SUM159 cell line to palmitate. A combination of palmitate and oleate attenuate this decrease viability. Moreover, the overexpression of CD36 itself increases free fatty acids storage in our two models in 2D and in 3D as well as the migratory and invasive capacities of the clones derived from the more aggressive breast cancer cell line but not of the clones derived from the less aggressive one.
Secondly, we then evaluated ferroptosis following palmitate treatment and found an increase lipid peroxidation as well as downregulation of anti-ferroptotic genes in the SUM159 cell line but not in the MCF7. Interestingly, the overexpression of CD36 seems to amplify this effect with higher lipid peroxidation and ferroptosis-related genes expression. Finally, the combination of oleate, known to limit the peroxidation of lipid bilayer led to a decrease lipid peroxidation and ferroptosis in the most aggressive breast cancer cell line.

Conclusion
Our results suggest an important role of CD36 in the aggressivity of breast cancer cells as well as in increasing vulnerabilities of those cells to ferroptosis thus opening potential new therapeutic approaches promoting ferroptosis in the most aggressive breast cancers.

Keywords : Breast cancer, CD36, metabolism, ferroptosis

P15 - DUAL ROLE OF FERRITIN IN FERROPTOSIS INDUCTION: When a hero becomes a villain

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The evolution of the complex form of lives on Earth occurred thanks to the rise in atmospheric oxygen level. This evolutionary jump came with the price tag that is paid by living organisms until this day. The rise in atmospheric oxygen rendered iron acquisition problematic due to precipitous decline of its bioavailability. On the other side, the level of free iron had to be kept in check, due to its high reactivity/toxicity in oxidized circumstances that can lead to specific type of cell death, now known as ferroptosis. Both problems seem to be overcome by the evolution of ubiquitous iron storage system known as ferritin, which thus could be seen as fundamental for evolution of aerobic life. Nonetheless, recent literature suggests that ferritin and its turnover (ferritinophagy), are key players of ferroptosis. It remains unclear how and under what circumstances this protective complex becomes threatening to the cell, as well as whether its targeting could be used in the context of anticancer treatment.
To investigate this issue, we generated two knockout medulloblastoma cell lines: 1) one that has genetically deleted heavy chain of ferritin (FTH-/-), and thus no ability to form functional iron storage; and 2) second lacking the gene for a cargo receptor nuclear receptor coactivator 4 (NCOA4-/-), fundamental for degradation of ferritin, which is, thus, unable to use existing iron storages. Both cell lines show no signs of cell death in control conditions. Maintenance of the homeostasis seems to be insured by the changes at the molecular level, most notably changes at the level of iron transporters. Nonetheless, the prominent difference between the cell lines has been observed upon changed iron flux, where FTH-/- showed the highest and NCOA4-/- the lowest sensitivity to iron fluctuation. We confirmed that the cell death observed upon iron overload was ferroptotic in nature, as it was preceded by significant accumulation of lipid hydroperoxides, the most prominent hallmark of ferroptosis, and it was completely prevented by addition of a ferroptotic inhibitor – ferrostatin 1. Kinetic analysis of iron-induced cell death in WT showed that initially, iron overload induced increase in ferritin content, while later (6h of treatment) this increase was accompanied by the rise in NCOA4 protein level, which most likely triggers the degradation of the ferritin, release of free iron and ferroptosis execution.
The results obtained in the study illuminated the dual role ferritin plays in ferroptosis sensitivity. Namely, the ferritin as an iron-buffering system can be seen as anti-ferroptotic molecule, while if its storage capacity is exceeded and once it starts to breakdown, ferritin can be classified as a pro-ferroptotic molecule. Considering high dependency of the cancer cell on iron, targeting iron storage represents a potential approach to crippling a key cancer dependency. Furthermore, this seems to be of particular importance for cancer stem cells, not so long ago identified as responsible for resistance and relapse, which appear to rely on iron until “to Die for” it.

Keywords : iron, ferroptosis, medulloblastoma, ferritin, NCOA4

P16 - Effects of the interactions between GSCs and MSCs on GBM progression and resistance to chemotherapy

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Introduction. Glioblastoma (GBM) is the most aggressive brain cancer, with short patient survival. The standard treatment for glioblastoma is maximal safe resection followed by radiotherapy and several cycles of Temozolomide (TMZ) chemotherapy. However, glioblastomas usually recur within few months. The exact mechanisms underlying this resistance are still partly unknown. GBMs contain self-renewing, glioblastoma stem cells (GSC) which contribute to the recurrence of the disease. The glioblastoma microenvironment is highly heterogeneous and notably contains astrocytes and mesenchymal stem cells (MSCs). The laboratory has shown that these cells can form tunneling nanotubes connections with GSCs, permitting the transfer of mitochondria to GSCs. We have demonstrated that the acquisition of MSC mitochondria increases TMZ resistance in GSCs.
Our goal is to investigate how does this mitochondria transfer contribute to the increased GSCs resistance to TMZ.

Materials and methods. The laboratory protocol of Mitoception allows the quantitative transfer of pre-isolated MSCs mitochondria to GSCs. Isotopic profiling was performed, followed by metabolomic analysis to track glucose and glutamine in the GSCs metabolism. The metabolites and proteins produced by GSCs following the acquisition of MSCs mitochondria were detected respectively by NMR spectroscopy and proteomic analyses of conditioned media.

Results. Isotopic profiling showed that the acquisition of MSC mitochondria induces a higher orotate turnover in GSCs. The inhibition of orotate production with Brequinar, restored the TMZ sensitivity of GSCs. As expected, NMR spectroscopy showed an increase in lactate secretion in the GSCs after acquisition of MSCs mitochondria, in agreement with the high glycolysis in these cells observed by the laboratory in previous studies. Among the other metabolites secreted by the GSCs, alanine was more secreted as well after MSCs mitochondria acquisition. Proteomic analysis showed, some variation at the protein level in the GSCs supernatant, further experiments are being conducted.

Conclusion. Mitochondria transfer from MSCs confers chemoresistance to GSCs through high orotate turnover. We are now studying the effect of the detected variations in the metabolites and proteins secretion on the GBM progression and resistance to TMZ. We expect that mitochondria transfer may be involved in other mechanisms that could support GSCs resistance to TMZ.

Keywords : Glioblastoma Stem Cell, Mesenchymal Stem Cell, Mitochondria, Chemoresistance, Metabolism

P17 - CD36 drives metastasis and relapse in acute myeloid leukemia

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Introduction
Identifying mechanisms underlying relapse is a major clinical issue for effective cancer treatment. The emerging understanding of the importance of metastasis in hematological malignancies suggests that it could also play a role in drug resistance and relapse in acute myeloid leukemia (AML).
Using AML patient-derived xenografts treated with cytarabine (AraC), we have previously shown that CD36, a plasma membrane receptor and fatty acid transporter, was among the most strongly upregulated genes in chemoresistant cells (Farge et al., Cancer Discovery, 2017).
In this study, we investigated the clinical relevance of this observation, and the mechanisms by which CD36 expression may contribute to chemoresistance and relapse.

Methods

A cohort of 1,273 AML patients from the Toulouse University Hospital (TUH) was examined to evaluate the association between CD36 expression and various clinical outcomes. The roles of CD36 in lipid metabolism, blast migration and senescence were studied. Specifically, the interaction between CD36 and its ligand thrombospondin-1 was assessed. Finally, xenograft mouse models were used to evaluate the therapeutic potential of CD36 invalidation.

Results
In our cohort of 1,273 AML patients, we uncovered that CD36 was positively associated with extramedullary dissemination of leukemic blasts, increased risk of relapse after intensive chemotherapy as well as reduced event-free and overall survival. CD36 was dispensable for lipid uptake but fostered blast migration through its binding with thrombospondin-1. CD36-expressing blasts, which were largely enriched after chemotherapy, exhibited a senescent-like phenotype while maintaining their migratory ability. In xenograft mouse models, CD36 inhibition reduced metastasis of blasts and prolonged survival of chemotherapy-treated mice.

Conclusion
CD36 promotes blast migration and extramedullary disease in AML. Our results pave the way for the development of CD36 as an independent marker of poor prognosis and a promising actionable target to improve the outcome of AML patients.

Keywords : Acute myeloid leukemia, metastasis, CD36

P18 - Mitochondria transfer induces extracellular matrix remodeling in Glioblastoma

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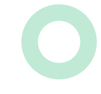
Introduction. The tumor microenvironment (TME) is highly dynamic and plays a significant role in cancer progression. The crosstalk between tumor and non-cancerous cells of the TME occurs through different mechanisms including the recently described tunneling nanotubes (TNTs). TNTs allow the intercellular exchange of various cargos, including mitochondria, which results in tumor progression and therapy resistance.
Glioblastomas (GBM) are aggressive brain tumors whose resistance to therapy is supported by the high tumor heterogeneity and plasticity and the presence of glioblastoma stem cells (GSCs). Cells of the TME, such as mesenchymal stem cells (MSCs) and astrocytes, also support GBM progression.
Our laboratory and others have shown that MSCs and astrocytes can transfer mitochondria to GSCs. We have shown that MSC mitochondria contribute to increased GSC resistance to temozolomide (TMZ) through major metabolic reprogramming. Our current goal is to identify other mechanisms allowing mitochondrial transfer to contribute to GBM progression.

Materials and methods. We used our protocol of Mitoception, which allows the quantitative transfer to target cells of pre-isolated mitochondria, to determine their effects in GSCs. We analyzed by RNAseq how mitochondrial transfer alters GSCs gene expression. We also developed a model of 3D spheroids of human GSCs, either alone or in coculture with human MSCs or astrocytes, which were analyzed by either live imaging or cryosections microscopy.
Results. RNAseq analyses showed that GSCs transcriptional response to TMZ is modified upon acquisition of MSC mitochondria. Gene expression in relation to ECM composition was also altered. Confocal microscopy of GSCs organoids showed the formation of TNT-like structures. Imaging of the GSC spheroid showed the effects of mitochondrial transfer, both from MSCs and astrocytes, on the spheroid ECM structure, as shown by second harmonic generation (SHG) and PSR imaging under polarized light.

Conclusion. Mitochondria transfer to GSCs induces changes in the ECM as shown in GSC spheroids. The effects of these changes on GSC invasive capacities are currently being investigated.

Keywords : glioblastoma stem cells, mitochondria transfer, tumor microenvironment, extracellular matrix



P19 - Exploring the role of the extracellular matrix as a nutrient source to fuel breast cancer cell metabolism

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Cancer metabolism is one of the hallmarks of cancer where cancer cells have a different metabolic behaviour than normal cells. Due to the high tumour growth rate and the limited blood supply, the tumour microenvironment has been found to be hypoxic and deprived of nutrients. Therefore, cancer cells can reprogram their metabolism and adopt new strategies to survive. One of these strategies is to uptake macromolecules from the tumour microenvironment, degrade them and use them as source of nutrients.
The tumour microenvironment consists of plenty of cells in addition to the extracellular matrix (ECM) which is a collection of extracellular molecules secreted by cells.
In this project, the role of ECM in breast cancer metabolism will be studied. The proposed study will address the role of ECM internalisation in breast cancer growth under starvation conditions.
To investigate the role of ECM in breast cancer metabolism, MCF10-CA1 cells, which are highly invasive breast cancer cells, were plated on different types of ECM and the cells proliferation was quantified under starvation conditions. The cells proliferation was also quantified under starvation conditions after inhibiting ECM uptake. The results show that the proliferation of the cells was enhanced after plating the cells on ECM under starvation conditions comparing to on plastic and inhibiting collagen I uptake reduced this effect. In addition to that, inhibiting integrins could significantly reduce the effect of ECM on breast cancer cells proliferation under starvation conditions. So far, we found that highly invasive breast cancer cells are able to use ECM as source of nutrients to survive under starvation.

P20 - LIPG and cholesterol homeostasis in breast cancer

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In breast cancer, the endothelial lipase or lipase G (LIPG) is highly expressed in a limited subset of tumours and significantly associated with shorter metastasis-free survival in node-negative, untreated patients (Cadenas et al., 2019). LIPG is a cell surface-associated protein with multiple actions on serum lipoproteins that influence the cellular lipid profile. Silencing LIPG in breast cancer cells was accompanied by less migration and slower cell adhesion to extracellular matrix. Loss of LIPG led to expression changes in cholesterol and lipoprotein metabolism genes. Most prominent changes were observed for SCARB1, and ABCG1, both downregulated. We have identified a role of LIPG in tumor cholesterol homeostasis via regulation of cellular cholesterol exchange with lipoproteins. Since cholesterol metabolism plays a role in tumor cell metastasis, LIPG may promotes processes related to metastasis.

Keywords : LIPG, Cholesterol, Lipoproteins, Metastasis,

P21 - IκBα facilitates metastasis formation in lung cancer through endothelial activation

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Introduction
Lung adenocarcinoma (LUAD) stands as the leading cause of global cancer-related mortality, accounting for approximately 1.6 million deaths annually and nearly matching its incidence rate. Metastasis remains the vulnerable point of cancer treatment, primarily responsible for fatalities. Among the key components of the tumor microenvironment (TME), Endothelial Cells (ECs) serve as both significant players and the initial hurdle that cancer cells must overcome to reach distant metastatic sites.

Material and Methods
Model of IκBα overexpressing lung cancer cells in a panel of preclinical models of human were used in vitro and in vivo to validate the impact of IκBα overexpression in lung cancer progression. Tumor ECs were modeled using tumor-conditioned HUVECs.

Results and Discussions
IκBα-overexpressing lung cancer cells displayed increased proliferation, colony formation as well as the Lung cancer cells overexpressing IκBα exhibit heightened proliferation, colony formation, migration capabilities, and an increased propensity for metastasis formation, while also displaying reduced apoptosis. Our study further unveils that the secretome of IκBα-overexpressing lung cancer cells augments the expression of endothelial adhesion molecules like intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). This, in turn, fosters cancer cell adhesion and transmigration through ECs. Furthermore, our investigation highlights the significant glycolytic activity observed in IκBα-overexpressing lung cancer cells. This heightened glycolysis potentially prompts a metabolic adaptation in ECs by activating them. Remarkably, pre-treatment of lung cancer cells with 2-Deoxy-D-glucose (2DG), a glucose analog targeting cancer cell energy metabolism, negates the proadhesive impact of the cell's secretome on ECs. Notably, 2DG also exhibits substantial potential in inhibiting specific cancer cell growth, and its combination with other therapeutic agents or radiotherapy demonstrates a promising synergistic anticancer effect.

Conclusion
In summary, our study characterizes the activation of IκBα signaling in lung cancer as a pivotal contributor to metastasis, presenting a viable target for therapeutic intervention.

Keywords : Lung Cancer; Microenvironment ; Endotelial Cells; Mitochondria; I?B?;

P22 - Functional mitochondrial respiration is essential for glioblastoma formation

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Introduction: In recent years, horizontal mitochondria transfer (HMT) has been shown to take place between mammalian cells in vitro and in vivo in various experimental set-ups both under physiological and pathological conditions including cancer. Using in vivo subcutaneous tumour models of melanoma and breast cancer, our laboratory demonstrated that transport of healthy mitochondria from tumour stroma to neoplastic cells can support their proliferation and increase their tumour-forming ability (Bajzikova et al., 2019). However, little is known about the role of HMT in the context of brain cancer.

Methods: To investigate HMT within the intact environment of the brain, we used orthotopic syngeneic brain cancer mouse models. We grafted 50 000 cells of glioblastoma GL261 cell line into the caudate putamen of C57Bl/6 mice. Two types of GL261 cells were grafted, either parental cells with intact mitochondrial DNA (mtDNA) or rho0 cells with depleted mtDNA. We also derived cell lines from tumours formed from rho0 cells at different stages of tumour formation and used them for further experiments.

Results: We observed a significant delay in the formation of tumours from rho0 cells compared to parental cells. Based on our results, the delay was caused by the need of mitochondrial acquisition by rho0 cells to meet the bioenergetic needs for tumour growth. mtDNA sequencing revealed that cells without mtDNA acquired mitochondria from the host after engraftment into the brain. Moreover, these cells restored mitochondrial cristae structure, mtDNA levels, transcription of mtDNA-coded genes and formation of respiratory supercomplexes, resulting in mitochondrial respiration recovery and accompanying lower levels of glycolysis.

Conclusion: Using a model of respiration-defective cells, we showed that HMT occurs in glioblastoma in vivo leading to recovered respiration, which is required for brain tumour formation. Our results suggest that the limiting factor for glioblastoma formation and progression is dihydroorotate dehydrogenase-driven pyrimidine biosynthesis, which requires functional respiratory chain that is malfunctional in rho0 cells.

Keywords : mitochondria, mtDNA, glioblastoma, respiration

P23 - Engineering obesity microenvironments to investigate breast cancer progression and invasiveness.

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Obesity is considered a high-risk factor for breast cancer, as it is linked with higher mortality rates and increased levels of recurrence compared to lean individuals. Recent studies have reported that it promotes tumour initiation and progression by providing a specific microenvironment with suitable mechanical and architectural cues. Nevertheless, obesity also causes chronic inflammation and altered metabolism that both play a role at different stages of breast cancer. In this study, an in vitro model is developed to investigate the effect of these distinct obesity-associated conditions on tumour progression and invasion.

The extracellular matrix (ECM) is a key fibrillar/scaffolding component of physiological and pathological tissues. It was reported that ECM composition, microarchitecture and mechanics all play essential roles in the cross-communication and spatial interaction between obese and cancer tissues. In this study, we have used temperature casting to engineer obese-mimicking and control/lean collagen scaffolds with tuneable/controlled microarchitecture and mechanics. First, the interaction between those obese and lean microenvironments with cancer cells was assessed by monitoring both the growth (and invasion) of embedded tumour spheroids and the associated structural alterations of the surrounding collagen/ECM scaffolds. Next, the effect of inflammation and altered metabolism was explored by treating the tumours embedded in obese and lean mimicking scaffolds either with adipocyte-conditioned media (ACM) or TNFα. Finally, in vitro differentiated adipose cells were incorporated in the tumour spheroids to assess the role of direct cell-cell contact in invasion to the adipose tissue.

Our data indicate that (i) in absence of obese-related biochemical cues, cancer cells' invasion depends primarily on matrix architecture (mesh size and fibre thickness), (ii) ACM treatment has more effect than TNFα treatment on tumour growth and this effect is more dramatic in obese-mimicking environments compared to lean ones, (iii) although the presence of adipose cells seem to slow down tumour progression at early time points, invasion proceeds at later timepoints when adipocytes undergo a change in phenotype. Those results pinpoint the intricate contributions of ECM material properties and biochemical components of the obese-associated tumour microenvironment. They also suggest that the phenotypic change of the adipose cells in direct contact with tumour spheroids might facilitate the 'recruitment' of more adipose cells by cancer cells and facilitate tumour invasion.

Collectively, our findings establish a substantial and complex role of obesity markers (and adipose tissue in general) in breast cancer progression and invasion. Following up on this data is needed, as it will allow us to unravel the link between obesity and breast cancer metastasis, recurrence, and mortality rates.

P24 - Contribution of the peripheral nervous system during perineural invasion of lung adenocarcinomas

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Perineural invasion (PNI) is defined as the colonization of cancer cells into nerve fibers. This process leads to tumor recurrence, distant metastasis and poor prognosis. PNI has been detected in several cancers such as head and neck, pancreatic and colorectal cancers for which underlying molecular mechanisms start to be identified (1). PNI was recently described in post-resected non-small cell lung cancer (2) but has not been deeply investigated yet. In this context, our project aims to better characterize the molecular mechanisms underlying PNI in lung adenocarcinoma (LUAD).
In LUAD, a study identified that lung cancer cells increase CXCL5 expression in the glial myelinating cells associated with nerves named Schwann cells. CXCL5 in turn triggers cancer cell migration through PI3K/Akt/GSK-3β signalling as also described in pancreatic cancers (3). Furthermore, a meta-analysis of LUAD transcriptome highlighted that Schwann cells associated with lung tumours harbour a dedifferentiated phenotype to facilitate cancer cell migration toward nerves (4). The meta-analysis also identified an accumulation of alanine/glutamate and p53 signalling hyperactivity in dedifferentiated Schwann cells. However, the role of peripheral nerves in tumour progression and the signaling pathways that trigger nerve colonization by cancer cells remain largely understudied in LUAD.

Recently, we uncovered that the metabolic kinase and tumor suppressor LKB1 is a key player to establish and maintain sensory neurons and Schwann cells of the peripheral nerves from bipotent progenitors (5). Using genetically engineered mouse models to spatiotemporally inactivate Lkb1 in these progenitors, we identified that LKB1 fine-tunes alanine and glutamate levels in a coupled manner with mTOR activity in the glial lineage, while limiting oxidative stress and p53 signaling earlier during development (6, 7). These results on alanine/glutamate levels and p53 signalling are similar to those of the meta-analysis of LUAD transcriptome.
Taking advantage of our genetic models, we now investigate if peripheral nerves and LUAD cells communicate to modulate cancer cell

dissemination. We currently explore if this communication requires LKB1 signaling in nerve cells. By using cultures of dorsal root ganglion explants as an ex vivo 3D model of the perineural niche, we showed that molecules released by nerve cells activate the proliferation of lung cancer cells as spheroids and maintain their epithelial profile. We also observed that physical proximity of nerve cells is critical to limit the migration of lung cancer cells. Interestingly, we found that LKB1 loss in nerve cells removes this limitation and triggers a mesenchymal profile. Using transcriptomic and proteomic techniques, we investigate the different signaling pathways activated by the peripheral nervous system under the control of LKB1 and involved in the aggressiveness of lung adenocarcinomas. Based on our findings, we also aim to perform a screening of chemical compound libraries to identify new molecules modulating tumour properties. At last, we have started the clearing of lungs with tumours for 3D in vivo visualization of nerve invasion by tumour cells.
Overall, this project thus characterizes the molecular mechanisms underlying perineural invasion in lung adenocarcinomas to identify novel therapeutic targets.

Keywords : Nerve cells, LUAD, perineurial invasion, LKB1

P25 - Unraveling the role of mitophagy during lung cancer progression

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Lung cancer is a leading cause of death worldwide. While recent major advances were made in the treatment of this disease, the overall 5-year survival rate is only 15%, reinforcing the need of innovative and more efficient therapeutic strategies.
Mitophagy, a selective form of autophagy involved in the elimination of damaged mitochondria, is a major process that enables cancer cells to resist both environmental stress and chemotherapeutic agents. It has also recently been established that mitophagy plays an essential role in the regulation of inflammatory responses.
Knowing both the importance of autophagy in cancer initiation and progression, and that lung cancer is highly dependent on mitochondrial metabolism, we first analyzed the level of mitophagy in a genetically engineered mouse models of lung adenocarcinoma to study mitophagy *in vivo*. We therefore breed *Kras*$^{LSL-G12D}$ deficient or not for p53 (KP and K model respectively) with Mito-QC mice, which express a pH-sensitive fluorescent mitochondrial tandem, allowing assessment of mitophagy and mitochondrial architecture. Using this unique model, we monitored mitophagy upon lung cancer development by measuring Mito-QC fluorescence at different stages of lung tumor (healthy, pre-malignant lesions or established tumors). Our data indicate that mitophagy is increased in all pre-neoplastic lesions, whereas this induction is more heterogenous at the adenocarcinoma (ADK) stage, even though the oncogenic driver mutations are the same. Finally, we used an innovative intratracheal injection of Cre recombinase-expressing lentivirus with a specific guide RNA to invalidate the expression of two key auto/mitophagy genes (ATG7 or Pink1) in vivo. Overall, our study highlights the impact of mitophagy on lung cancer development and immune cell infiltration.

Keywords : Lung cancer, mitochondria, autophagy, tumor immunity, mouse model

P26 - UPR activation in cancer cells promotes anti-cancer immune response through DPM1-dependent control of PD-L1 glycosylation.

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The tumor microenvironment (TME) is characterized by hostile conditions that lead to the induction of the unfolded protein response (UPR) in both cancer cells and immune infiltrating cells. However, how the UPR in cancer cells can modulate the anti-cancer immune response remains to be discovered. To elucidate this effect, we performed a characterization of the IRE1-alpha interactome using a proximity-dependent biotinylation-based approach (BioID). Among the different partners identified, we showed that DPM1, a key player in proteins N- and O-glycosylation, controlled the immunogenicity of tumoral cells (using colon and lung cancer models). Indeed, we demonstrated in hot and

cold tumor models that DPM1 KO prevented tumor growth only in the presence of a functional adaptive immune system (as depleting cytotoxic CD8+ T cells prevented the protective effect). Mechanistically, DPM1 KO leads to reduced production of key cytokines and cell surface expression of PD-L1 (a major player in the control of adaptive immune response) through ERAD (ER-associated protein degradation). Additionally, DPM1 KO in cancer cells promotes M1 macrophages polarization overcoming immunosuppression and enhances cytotoxic T cell activity. Thus, our work reveals how tumoral UPR can limit tumor growth and suggests that DPM1 inhibition is a useful strategy for improving cancer immunotherapy.

P27 - Aspartate synthesis and secretion in glucose-deprived lung cancer and normal lung epithelial cells

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Introduction and objectives: Glucose levels are frequently reduced in solid cancers including lung cancer, due to rapid consumption and poor vascular supply. On the contrary, other metabolites, like aspartate, may be enhanced in the tumor microenvironment (1). Here we addressed the metabolic adaptations in lung cancer cells facing glucose limitation.

Methods: Stable isotopic labeling followed by nuclear-magnetic resonance (NMR) spectroscopy or mass spectrometry was performed in lung cancer cell lines and normal bronchial epithelial cells.

Results: When A549 lung cancer cells were supplied with 13C5-glutamine in low glucose (0.2 mM) compared to high glucose (10 mM) conditions, enhanced 13C-labeling of aspartate and release of labeled aspartate to the medium were observed. The low glucose-induced release of aspartate could be observed in different lung cancer cell lines, but also in normal bronchial epithelial cells. Expression levels of aspartate synthesis enzymes including aspartate aminotransferase (GOT1 and GOT2) were inconsistently changed. However, a significant decline of reduced versus oxidized nicotinamide adenine dinucleotide (NADH/NAD+) was found in low glucose conditions. NADH is formed during glycolysis and the electrons are translocated to the mitochondria via the malate-aspartate shuttle, involving the synthesis of aspartate by (mitochondrial) GOT2 and further conversion to oxaloacetate and malate in the cytoplasm. Crispr-Cas9 mediated knockout of GOT2 suppressed aspartate synthesis, showing that the proximal arm of the malate-aspartate shuttle was active. However, the subsequent reactions may be halted due to a decline in NADH caused by a suppression of glycolysis, leading to aspartate accumulation and release. Indeed, restoring NADH by exogenous lactate clearly reversed low-glucose induced aspartate synthesis and secretion. Hypoxia, which affects (tricarboxylic acid) TCA cycling, significantly reduced aspartate formation. Interestingly, an enhancement of cellular aspartate production caused by NADH loss has been recently shown upon pharmacological inhibition of glycolysis (2). NADH is required by the (reverse) GAPDH reaction in gluconeogenesis. The initial step of gluconeogenesis, mediated by phosphenolpyruvate carboxykinase (PCK2) is activated in glucose-starved lung cancer cells, as found by our and other groups (3,4). We show that the reverse GAPDH reaction shuttles carbons from glutamine to ribose-phosphate synthesis in low glucose conditions. Thus, NADH consumption by GAPDH (running in the reverse direction) may contribute to NADH decline and further enhance aspartate release.

Discussion and conclusion: Aspartate is released into the extracellular space by both, lung cancer and normal lung cells in low glucose conditions and the release is linked to a switch from NADH forming glycolysis to NADH consuming gluconeogenesis. Aspartate secretion in low glucose containing regions of the tumor may therefore contribute to shaping the acellular tumor microenvironment.

Keywords : Gluconeogenesis; lung cancer; starvation; malate-aspartate shuttle

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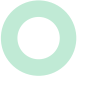

P28 - Biomechanical reprogramming by DDR1/2 collagen receptors in cutaneous melanoma: Impact on tumor cell metabolism and therapeutic response

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Cutaneous melanoma is a highly malignant and invasive skin cancer. Despite successful therapies targeting the BRAFV600E oncogenic pathway or immune checkpoints, resistances occur. Upon microenvironment and therapeutic pressures, melanoma cells can switch from melanocytic differentiated states to dedifferentiated states associated with increased expression of receptor tyrosine kinases and mesenchymal markers (1-4). Such adaptive plasticity was described as a driver of resistance to targeted therapy. We previously described that dedifferentiated cells can acquire extracellular matrix (ECM) remodeling activities and that tumor exposure to BRAF inhibitors induces tumor stiffening associated with increased ECM deposition and signaling (5,6,7). Additional experiments showed that ECM signaling on melanoma cells involves the collagen receptors discoidin domain receptors 1 and 2 (DDR1/2), which activate a NIK/NFkB2 pathway involved in therapeutic resistance (7). Our recent analysis of melanoma cells cultivated on soft or stiff collagen matrices showed that DDR are involved in collagen stiffening-induced proliferation, invasion and drug resistance of dedifferentiated melanoma cells, through the actomyosin/YAP pathway. This indicates that the dedifferentiated tumor cell state is addicted to ECM mechanical signals. However, how melanoma cells rewire their cellular metabolism to support survival advantage on stiff collagen matrices in the presence of BRAF-targeting therapies remains unknown. Here, using metabolomics analyses, we discovered that extracellular mechanical signals differently modulate the metabolism of differentiated and dedifferentiated melanoma cells. Interestingly, we also demonstrated that collagen rigidity affects both the mitochondrial dynamics of dedifferentiated cells and their lipid storage capacities. Importantly, we found that metabolic rewiring induced by collagen stiffening is dependent on DDR1/2 expression in dedifferentiated cells. Electron microscopy analysis confirmed the action of DDR1/2 on ECM stiffness-induced mitochondrial fusion. Interestingly, exposure of melanoma cells to targeted therapy modulated mitochondrial dynamics and increased fusion, a phenotype repressed when DDR1/2 are depleted by RNAi. Taken together, this study establishes an original link between collagen receptors DDR1/2 and metabolic reprogramming of aggressive dedifferentiated melanoma cells adapting to mechanically distinct microenvironments and targeted therapy pressure.

Keywords : Cutaneous melanoma, Metabolism, Extracellular matrix, Mechanotransduction, Therapeutic resistance

P29 - METABOLIC ANALYSIS DURING LUNG TUMOR SPHEROID GROWTH IN DIFFERENT MATRIX RIGIDITIES

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Introduction
Cancer is one of the leading causes of death in the world and the search for its cure is the main goal of a large percentage of researchers worldwide1. There are different approaches for cancer research and in this study, we have focused on three-dimensional (3D) cell cultures. Most research in cancer biology is based on experiments with two-dimensional (2D) cell cultures in vitro, but this type of culture presents many limitations, which has led to the development of 3D cell cultures that allow a better recreation of cancer biology2. This method represents more faithfully the physiological conditions of tumor formation, so we aim to recreate the 3D spherical shape in vitro, with a microfluidic system, which will allow us to perform 3D cell cultures on a small scale with reasonable control of the environment3. Using these models, we have studied the metabolism of tumor cells and how variations in matrix stiffness affects. We have focused on determining the relationship between the stiffness of the microenvironment and glucose metabolism as the main nutrient.

Materials and methods
A variety of microfluidic, microscopy and image analysis and 3D cell culture techniques were used in this work.
The microfluidic devices were made of PDMS, with a central chamber containing a hydrogel based on collagen type I that mimics matrix for cell culture and two side channels through which nutrients are introduced4. The cells used were A549 lung tumor and were monitored with a phase contrast microscope. For the quantitative study of cell proliferation, the alamarBlue® Cell Viability Reagent was used. The analysis of daily culture medium to determine the amount of glucose was performed with a Ultra Performance Liquid Chromatography (UPLC). Finally, different stains were used to study the structure of the spheroids, which was visualized by confocal microscopy.

Results
In this study, the consumption of glucose per cell was analyzed at different concentrations of this nutrient, including a comparison between different stiffnesses of the microenvironment. The stiffness was modified by changing the collagen concentration. In this way, we have observed that the behaviour of the tumor cells varies according to both the nutrient availability and the matrix stiffness. These variations were observed during spheroid morphogenesis in both cell proliferation and glucose consumption.

Conclusion
Most existing 3D models of lung cancer are based on cell clusters already formed by the accumulation of cells5. However, this work proposes a novel model that encompasses the entire process of tumor morphogenesis, growth and maintenance guided by the surrounding matrix, allowing all stages to be studied. The results obtained highlight the importance of the extracellular matrix, establishing a relationship between its stiffness and the metabolism of cancer cells, which is also reflected in tumor progression. This may provide new insights of how matrix stiffness can regulate cancer development and progression, which can be used to design new therapies.

Keywords : Metabolism, glucose, stiffness, spheroid, hydrogel

P30 - Metabolic dialogue between mammary adipocytes and tumor cells: role in the aggressiveness of a specific metabolic subtype of triple-negative breast cancers

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Among breast cancers, triple-negative breast cancer (TNBC) remains an unmet medical challenge due to its aggressiveness and the absence of targeted therapies. Recently, TNBC samples have been classified into three heterogeneous metabolic-pathway-based subtypes (MPSs) with distinct metabolic features [1]. The group with the worst prognosis (MPS2) exhibits an increased capacity to uptake exogenous free fatty acids (FFA). Our hypothesis is that the interaction of these cells with tumor-surrounding mammary adipocytes (the main source of FFA in the tumor microenvironment [2]) is a key event in their aggressiveness.
We used a recently described model of co-culture, established by our team [3], between human isolated mammary adipocytes (M-Ad) grown in 3D matrices and human cell lines representative of the 3 MPSs to investigate: i) the transfer of lipids between M-Ad and the different MPS tumor cells using immunofluorescence and lipidomic approaches ; ii) the consequences of this lipid transfer on metabolic remodeling (fatty acid oxidation (FAO) activity, Seahorse) and iii) tumor aggressiveness, including survival, proliferation, and chemoresistance, using real-time imaging.
When co-cultivated with adipocytes, the MPS2 group exhibits a higher accumulation of lipids (about 10-fold) than the other two groups, confirming our main hypothesis. The three MPS groups induce lipolysis in M-Ads at comparable levels, suggesting that the overexpression of FFA transporters is the cause of the higher lipid accumulation in MPS2 cells. In fact, we found that some transmembrane and intracellular lipid transporters (CD36, FATP3, FATP4, and FABP5) are overexpressed in MPS2 cells, and their function is being investigated using pharmacological and siRNA approaches. This transfer of lipids into the MPS2 cells leads to a metabolic remodeling favoring an increase in FAO activity, uncoupled from mitochondrial respiration and ATP production. Interestingly, MPS2 cells overexpress both CPT1a, involved in the transport of FFAs into mitochondria, and enzymes involved in FAO. This lipid accumulation and metabolic remodeling in co-cultivated MPS2 cells lead to increased survival (but not proliferation) and selective resistance to chemotherapeutic agents that induce oxidative stress and ferroptosis (e.g., doxorubicin). This effect is replicated by exposing MPS2 cells (but not the other MPSs) to oleate, a monounsaturated fatty acid (MUFA).
In conclusion, we have demonstrated that M-Ads specifically induce increased survival and chemoresistance in MPS2 cells. Our current hypothesis is that both reduced oxidative stress related to uncoupled FAO and changes in the balance between mono- and polyunsaturated FAs (in favor of MUFA) reduce ferroptosis, which is responsible for the observed phenotypical changes. Identifying key steps in this process, such as the involved lipid transporters, could lead to the identification of risk stratification markers and new pharmacological targets in these aggressive diseases.

Keywords : Triple negative breast cancer, adipocytes, metabolic dialogue, tumor progression, lipid metabolism

P31 - Colon cancer cells transform adipocytes into cancer-associated adipocytes

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Introduction: Tumor formation and progression depend on the interactions between cancer cells and their microenvironment (TME). Colorectal cancer (CRC) is located in the surrounding rich in adipose tissue. Adipocytes localized in the close proximately to tumor are named cancer-associated adipocytes (CAAs) and they are modified in comparison to those localized distant to tumor. CAAs might stimulate tumor progression (Grigoras and Amalinei 2023; Iacono et al. 2022). The purpose of our research was to examine the impact of CRC cells on

adipocytes transformation in vitro.

Materials and methods: We obtained CAAs using co-culture model. Three CRC cell lines were applied: two primarily (LS180 and HCT116) and the metastatic one (LoVo) which were seeded onto inserts and putted into plates with mature adipocytes for seven days. The expression of genes characteristic for adipocytes and involved in cellular metabolism were investigated via real time PCR analysis. Lipid content was quantified with Oil Red O staining and spectrophotometric analysis. Moreover presence of lipid droplets was visualized using immunochemical staining. We also used zymography to study the metalloproteinase activity in the conditioned media collected from adipocytes and CAAs and Western Blotting for verification of signaling cascades' activation.

Results: Our studies demonstrated that CRC cells reprogrammed adipocytes into CAAs. It was revealed as a reduction of the amount of lipids and alteration in adipocyte morphology and metabolism. Furthermore we observed decrease of the expression of genes characteristic for mature adipocytes: adiponectin, resistin and PPARγ (peroxisome proliferator- activated receptor γ) in cancer-associated adipocytes. Metabolic changes in CAAs in comparison to adipocytes were related to the reduced expression of genes involved in lipid metabolism like FAB4 (Fatty acid Binding Protein 4), LIPE (hormone sensitive lipase), perilipin 2, SC4MOL (sterol C4 methyl oxidase like protein), OSBPL9 (oxysterol binding protein like 9) and FADS1 (fatty acid desaturase 1). Moreover CAAs released higher amount of lactate, protease MMP2, and expressed increased level of ions transporters like MCT1 (monocarboxylate transporter 1) and NHE1 (Na/H + exchanger 1), as well as GLUT1 (glucose transporter 1) in contradistinction to adipocytes. Additionally, we noticed elevated level of phosphorylated Akt kinase and declined activation of ERK and STAT3 signaling pathways in adipocytes growing in co-culture in comparison to mono-cultured.

Conclusions: Adipocytes were converted into CAAs under co-culture with all tested CRC cell lines. Due to the reprogramming, adipocytes reduced their lipid content, probably because of the intensification of lipolysis, decreased of adipogenesis and the lower expression of genes involved in lipid metabolism. In addition, we noted higher level of MMP2 and lactate in the CAAs' medium in comparison to non-transformed adipocytes, as well as overexpression of the transporters of lactate, H+ ions and glucose in CAAs. This changes may trigger modifications in the tumor microenvironment and potentially promote cancer progression.

This research was funded by the National Science Centre, Poland, with a grant received by Dorota Nowak (OPUS 22, No. 2021/43/B/NZ3/01458).

Literature :
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P32 - Adipocytes support the aggressiveness of colorectal cancer cells

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Introduction
Since the global frequency of obesity is high, obesity-associated diseases are a rapidly growing problem of public health. Under normal conditions, adipocytes regulate multiple physiological processes, which in obesity due to excessive adipose tissue accumulation are dysregulated. This may be associated with different pathologies and worse outcomes in numerous types of cancer, including colorectal cancer (CRC), which is the third most common malignancy worldwide 1. As a consequence of the proximity of adipocytes and invasive cancer cells, adipocytes in the area of cancer display alterations and are termed cancer-associated adipocytes (CAAs) 2. The cross-talk between the CAAs and cancer cells might be increased in obesity, where the balance in secretion of molecules by adipose tissue is altered. Thus, our research aimed to analyze the adipocytes' involvement on CRC cells.

Materials and methods
In our study, we co-cultured adipocytes with CRC cells (lines LS180, HCT116, and Lovo) by applying the Transwell inserts model. The proliferation of CRC cells upon co-culture was tested with XTT, while their migration was evaluated by time-lapse microscopy and wound healing assay. The cell cycle was analyzed with a Flow cytometer, gene and protein expression by qRT-PCR, and Western blotting techniques, respectively. Lipid droplets were visualized by LipidSpot™488 staining and observed using a high-resolution confocal microscopy system (Leica Stellaris STED platform).

Results
We noted an increased proliferation rate of CRC cells co-cultured with adipocytes, that switched the distribution of cell cycle phases from G0/G1 into G2/M. Moreover, we detected improved migration and invasion abilities of CRC co-cultured with adipocytes. These cells also accumulated lipid droplets. Because lipid droplets could be synthesized de novo in cancer cells, we also evaluated the level one of the key enzymes participating in this process - fatty acid synthase (FASN). The identified changes in FASN amounts might at least partly explain the elevated content of lipid droplets in CRC cells cultivated with adipocytes. Furthermore, the level of glucose transporters GLUT1 and GLUT3, which mediate basal glucose transport, was increased in CRC co-cultured with adipocytes. In addition, we revealed modification in the glycosylation pattern of GLUT1 glycoprotein in CRC co-cultured with adipocytes.

Conclusion
Our results indicate that adipocytes are the crucial element of tumor microenvironment affecting cancer cell progression and the crosstalk

between adipocytes and CRC cells can disturb their biology.

1 Ye, P. et al. Cancers (2020)
2 Dirat, B. et al. Cancer Res. (2011)

This research was funded by the National Science Centre, Poland, with a grant received by Dorota Nowak (OPUS 22, No. 2021/43/B/NZ3/01458).

P33 - Bidirectional interaction between melanoma cells and adipocytes present in the tumor niche

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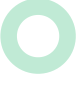
Introduction
One of the factors affecting the progression of melanoma is the tumor microenvironment, which consists of cellular elements, extracellular matrix, acidification, and a hypoxic state 1,2. Adipocytes, localized in the deepest layer of the skin, are also present in the tumor niche. Apart from lipid storage, they are also involved in inflammatory processes and hormone secretion. The aim of this research was to better recognize the bidirectional relationship between fat cells and melanoma, which remains not fully understood.

Methods
The influence of melanoma cells and adipocytes on each other was assessed by indirect co-culture system. The level of gene and protein expression was analyzed by qRT-PCR and Western blotting techniques, respectively. The fibroblastic phenotype of CAAs was confirmed by cell staining. The lipid content was measured by lipid detection using LipidSpot and by Oil Red O staining. Lactate secretion was established using a Lactate-Glo™ assay. Proteins secreted by CAAs were identified in cytokine and angiogenesis arrays. The proliferation of melanoma cells co-cultured with CAAs was assessed using an XTT proliferation assay.

Results
Obtained CAAs were identified by decreased levels of leptin, adiponectin, and resistin. Adipocytes co-cultured with melanoma presented fibroblastic features, such as increased levels of vimentin and TGFβRIII. Melanoma cells led to a reduction of lipid content in CAAs, possibly by downregulation of lipid synthesis pathways or enhancement of lipolysis. Adipocytes co-cultured with melanoma cells secreted higher amount of interleukin-6 and serpinE1 and less angiogenic molecules. CAAs also showed increased secretion of lactate and enhanced production of glucose, lactate, and ion transporters. Changes in adipocytes observed following melanoma co-culture resulted in an increased proliferation of cancer cells. Moreover, we observed elevated phosphorylation of STAT3, raised level of glucose transporters as well as downregulated MCT-1 expression in melanoma cells under the influence of CAAs. Epithelial-mesenchymal transition markers' expression was also raised in their presence, as well as the level of perilipin 2 and lipid content.

Conclusions
Melanoma cells led to decreased lipid content in CAAs, which might be related to enhanced delipidation or reduction of lipid synthesis. Fibroblast-like CAAs showed also metabolic changes. Observed phenomena may be the reason for accelerated proliferation of melanoma cells co-cultured with adipocytes. Moreover, under the influence of CAAs melanoma cells exhibited induced activation of signaling pathway, increased expression level of EMT markers, higher level of proteins involved in glucose metabolism and raised lipid content.

1 Mazurkiewicz, J. et al. (2021)
2 Dratkiewicz, E. et al. Cells 10, 1–26 (2021)
This research was funded by the National Science Centre, Poland, with a grant received by Dorota Nowak (OPUS 15, No. 2018/29/B/NZ5/00967).



P34 - A NOVEL CYTOSTASTIC INHIBITOR OF HYPOXIC PROSTATE CANCER CELLS

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Background: Hypoxia is a condition in which cells do not have enough supply of oxygen and is common in solid tumors. Prostate cancer (PCa), the second most common cancer in men, is also subjected to hypoxia. The hypoxic cells are resistant to radiotherapy and chemotherapy, metastasize, and thus have a very poor prognosis for patients. Hypoxia inducible factors (HIFs) are transcription factors that play a central role in detecting and adapting O2 level. They are heterodimers consisting of HIF-α and HIF-β. In mammals, HIF-α has different isoforms: HIF-1α, HIF-2α and less studied HIF-3α. HIFs help cancer cells adapt to hypoxic conditions and are involved in the establishment of treatment resistance. To date, only HIF-2α inhibitors have been tested and are currently in clinical trials. No specific HIF-1α inhibitor has yet been brought into the clinic. Thus, this project aims to characterize some novel HIF-1α inhibitors.

Methods: We tested compound A (comp. A) and compound B (comp. B) from marine sponge on human prostate epithelia P69 cell line, and on human PCa DU145 and PC3 cell lines. We tested effects of these compounds in hypoxia (1% O2) on HIF-1α stabilization (immunoblotting), nuclear localization (immunofluorescence), cell proliferation and viability, cell metabolism (glucose and lactate concentrations), and finally on gene expression (RNAseq). Based on the results of RNAseq, we also compared comp. B with docetaxel (DTX), a microtubule targeting drug that is used for cancer treatment including prostate cancer.

Results: Our results show that the comp. A destabilized HIF-1α protein, blocked its nuclear translocation but was too toxic for normal cells. Comp. B was able to destabilize HIF-1α, blocking its translocation to the nucleus and modifying the expression of HIF-1 genes. We observed a decrease in lactate production, leading to a reduction in cell proliferation in aggressive PC3 prostate cancer cells. RNASeq results showed that microtubule-related processes were affected. However, more destabilization and nuclear translocation of HIF-1a, but less condensed microtubules and induction of mitotic catastrophes were observed in the presence of comp. B compared to DTX when PC3 cells were cultured in hypoxia.

Conclusion: The compound B is a cytostatic-like inhibitor affecting microtubules differently from docetaxel, specific for hypoxic cancer cells, and may offer a novel therapeutic opportunity for prostate cancer.

Keywords : Hypoxia, HIF, prostate cancer

P35 - PDXO-on-chip: effect of microenvironmental conditions on their morphogenesis.

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Introduction: Pancreatic adenocarcinoma (PDAC) is a highly aggressive and deadly malignancy notorious for its late-stage diagnosis and limited treatment options, as well as its rapid progression and propensity to metastasize to other organs. Consequently, PDAC prognosis in patients has not improved over the years, leading to an overall five-year survival rate below 10%[1]. PDAC is characterized by a dense and complex stromal microenvironment, including cancer-associated fibroblasts, immune cells and a thick extracellular matrix, that take part not only in the disease's progression but also in chemo-resistance[2]. Accordingly, in vitro models development recreating PDAC microenvironment is essential for preclinical evaluation of novel therapies. Tumor-on-chip is an advanced platform for growing 3D models of tumors in controlled settings. It incorporates tumor cells and surrounding components in a microfluidic system[3]. We have focused on 'organoid-on-chip' (OOC), a cost-saving technology, that enable the examination of various parameters like matrix stiffness, pore size, and oxygen supply. OOC mimics organ architecture and function, offering a powerful tool for studying complex biological processes, disease modeling, drug screening, and personalized medicine.

Materials/Methods: Biological material: PDAC patient-derived xenograft organoids (PDXOs: 253, 215, 997). Microfluidic devices: single-chamber microchips for cells embedded in different matrices (BME, Collagen type I) and two medium channels. Quantitative PCR (qPCR). CK19 and H&E staining. Immunofluorescence. In vivo: orthotopic tumours and metastasis assay.

Results: H&E, CK19 staining and RNA analysis (SOX9, CFRT and KRT19) were performed to verify the correct generation of PDAC PDXOs. H&E revealed a classical cystic morphology with a lumen. RNA levels of CK19, SOX9 and CFRT were stable during the different

passages. In contrast with CK19 staining results in one of our PDXOs (253), which decreases in each passage, leading to the generation of two different organoid phenotypes. For this specific phenotype, H&E revealed a pseudostratified cell layer and compact complex inside. Apart from that, in vivo experiments were performed to assess PDXOs capacity to generate orthotopic tumours or metastasis compared to same 2D-cultered PDX. PDXOs gave rise to tumours faster (4 weeks vs 13 weeks) and produce liver macrometastasis in 2/4 contrary to 0/4 from 2D cultures.
After PDXOs characterization, we evaluated several microenvironmental conditions. Inside of our OOC, PDXO grows faster and bigger in hypoxia conditions. Nevertheless, when embedded in matrices, like BME or collagen type I, their behaviour strongly varies depending on the concentration. Higher collagen concentrations, with smaller pore size and increased stiffness, reduce the capacity of our organoids to grow. Importantly, stiffer conditions increase 253 compact organoids generation, which accumulate secretion products inside yet to be identified, due to stress response. Besides mechanical variables, other of our main aims is chemotherapy treatment. Organoid-on-chip combined three-dimensional and mechanical variables, which can influence response to therapy, mimicking better the tumour microenvironment. Indeed, sensitivity to the chemotherapeutic agent gemcitabine is greatly reduced in our PDXO-on-chip models when compared to 2D cultures.

Conclusion: Although further work is needed, our PDXO-on-chip model greatly reduces the experimental cost, allowing for real-time follow-up of therapy response in strictly controlled mechanical and metabolic conditions.

Keywords : PDAC, organoid, tumor stroma

P36 - The extracellular matrix influences pancreatic cancer cell sensitivity to chemotherapy by modulation of nucleotide metabolism.

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Introduction
Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy with an increasing incidence and a mortality rate projected to almost double by 2040 [1]. This dismal prospect arises from the absence of early symptoms, resulting in late diagnosis, and the failure of current therapies due in part to the presence of the so-called desmoplastic reaction that accompanies tumor progression [2]. The desmoplastic reaction is characterized by the overproduction and deposition of extracellular matrix components within the tumor niche, lowering the accessibility of chemotherapeutic agents, but also depriving cancer cells from oxygen and nutrients. Despite this hostile environment, cancer cells manage to maintain their growth and proliferation by adapting core metabolic processes to meet their energy demands. Recent reports suggest that the desmoplastic reaction not only functions as a physical barrier to protect the tumor, but also acts as a signaling platform and a nutrient pool that together may contribute to the metabolic plasticity of PDAC cells [3].

Materials and Methods
In this study, we set out to understand how the biochemical properties of the ECM shape the metabolic landscape of PDAC cells and how this ECM-mediated metabolic adaptation may modulate chemosensitivity. To that end, we established a 2D in vitro setting where PDAC cells are grown on a desmoplasia-mimicking bio-scaffold generated by cancer-associated fibroblasts (CAF), the main producer of extracellular matrix (ECM) and key inducer of desmoplasia in cancer. This experimental setting was coupled with a multi-omics integration analysis followed by an elegant metabolite tracing approach.

Results
We show that CAF-derived matrices (CDMs) promote significant transcriptional changes accompanied by extensive metabolic adaptations in PDAC cells that together result in enhanced tumor cell proliferation. This enhanced proliferation is coupled with a decreased sensitivity of PDAC cells to classical chemotherapeutic agents in association with transcriptional regulation of key elements of DNA repair mechanisms. Intriguingly, we found that the most impacted pathway in our observations was nucleotide metabolism, a substantially understudied metabolic process in PDAC, which has been proposed as a targetable vulnerability of cancer.

Conclusions
Our results couple desmoplasia with PDAC cell metabolic plasticity and suggest a link between purine/pyrimidine metabolism and DNA-repair-mediated drug resistance. Identification of the key players in this crosstalk will pave the way for the discovery of novel targets for combinatorial therapeutic strategies.

Keywords : pancreatic cancer, metabolism, extracellular matrix, nucleotide metabolism, chemosensitivity

P37 - Tissue metabolite composition driving metastatic organotropism in prostate cancer: the role of asparagine

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Prostate cancer (PC) is the second-leading cause of cancer-associated death in men. While good progress has been made in treating primary tumors, mortality in PC is still mainly caused by metastatic disease. Emerging evidence underlines the crucial role of tissue microenvironment in influencing metastatic cell distribution in distant organs and tumour-reforming ability. Specifically, PC preferentially metastasizes to bone and lung. Understanding the metabolic adaptations driving the organotropism of metastatic PC cells is crucial in developing novel therapeutic strategies for managing clinical metastatic diseases.
In the present study, we investigated the metabolic features driving metastatic colonization of PC cells preferentially in bone and lung.

The metabolite composition of different organs was analyzed by gas-mass spectrometry (GC-MS) on tissues collected from athymic healthy mice. The relevance of identified metabolites in sustaining metastatic niche colonization was then investigated in vitro by comparing normally adherent PC cell lines (two-dimensional-2D cultures) and cells grown in non-adherent conditions (3D cultures) as a model mimicking loss of extracellular support occurring during early stages of metastasis.

GC-MS Full Scan analysis of tissues from different organs of healthy mice revealed 28 common most enriched metabolites in bone and lung; among those, nine resulted biologically relevant, including asparagine.
Asparagine plays a critical role in tumour progression, being used in biosynthetic processes and supporting cell survival and proliferation under metabolic stressful conditions. In our in vitro model, exogenous supplementation of asparagine (but not aspartate) increases 3D cell growth. Adding asparagine to 2D cultures does not enhance cell proliferation nor increase cell migratory potential, indicating a specific role for asparagine in metastatic niche colonization. Further analysis underlined significant metabolic changes in cells adapted to non-adherent conditions. 3D cultures display lower mitochondrial oxidative metabolism than 2D cells. However, providing exogenous asparagine is sufficient to raise the oxygen consumption rate of 3D cells to the levels measured in adherent cells. 3D cultures also exhibit lower intracellular asparagine content and reduced expression of the enzyme asparagine synthetase (ASNS), which catalyzes the synthesis of asparagine from aspartate and glutamine. While 3D cultures are unaffected by glutamine absence in the growth media, 2D cell survival is strongly dependent on exogenous glutamine availability. However, providing asparagine rescues the survival of PC 2D cells under exogenous glutamine deprivation.

Finally, asparagine supports protein and nucleotide synthesis by signalling to the mammalian target of rapamycin complex 1 (mTORC1). Accordingly, asparagine supplementation in 3D cultures elevates the phosphorylation of mTORC1 and the activation of the downstream pathway through an aspartate-independent mechanism.
In conclusion, GC-MS analysis of tissues from different organs from healthy mice revealed that bone and lung are asparagine-rich environments, highlighting a possible influential factor driving PC metastatic organotropism. In in vitro models, asparagine supplementation favors the 3D growth of PC cells. Mechanistically, PC cells exploit asparagine to sustain their metabolism and activate the mTORC1 signalling pathway, further supporting cell growth, proliferation, and survival. Together, these data suggest that lowering asparagine availability in the bone and lung districts represents a promising strategy to target metastatic dissemination of PC cells.

Keywords : Organotropism, Prostate Cancer, Asparagine metabolism, microenvironment

P38 - Spatial metabolomics reveals a reprogramming of lipid metabolism by bacterial colibactin supporting immunosuppressive microenvironment in right-sided colon cancer

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Introduction: Intratumoral bacteria contribute to tumor heterogeneity through poorly understood mechanisms. It has been established that patients with right-sided colon cancer (RCC) exhibit a worse prognosis and differences in their tumor-associated biofilms and lipid metabolism when compared to left-sided colon cancer. However, it remains unclear whether and how the tumor-associated biofilm may influence the lipid metabolic reprogramming of the tumor microenvironment.

Materials and methods: To uncover the impact of Colibactin-producing by Escherichia coli (CoPEC) tumor-associated microenvironment and bacterial structure, 16S rRNA gene sequencing, metabolomic profiling in situ (7T-MALDI-FTICR), qPCR (to detect the presence of Colibactin) and RNA-sequencing/RNAscope were applied to RCC tumors. To confirm the human data, mouse and human colon carcinoma cells (MC38 and HCT116, respectively) were infected with the CoPEC clinical strain (11G5) or with its mutant strain that does not produce Colibactin (11G5ΔclbQ). Metabolomic in vitro results were confirmed using SpiderMass. Additionally, data were validated using a CoPEC infection on the MC38 mice graft model.

Results: By applying metabolomic profiling in situ, the presence of Colibactin-producing Escherichia coli (CoPEC) was identified to establish a high-glycerophospholipid microenvironment within RCC that bears oncogenic mutations in APC. Using spatial approaches, we revealed that bacterial microniches are poorly infiltrated by IFNγ-producing CD8+ T-cells. Notably, CoPEC infection leads to lipid droplet accumulation in MC38 and HCT116 cells. Interestingly, the aforementioned alterations in lipid metabolism positively correlated with immunomodulatory factors among which the human regenerating family member 3 alpha gene (REG3A). Herein, we revealed that engrafted tumors into Reg3b-deficient mice resulted in similar metabolic adaptation of the tumor together with a significant reduction in tumor growth after CoPEC infection. These data are supported by a decrease in Lpcat1 expression, a gene involved in the remodeling of glycerophospholipids, observed only in 11G5-infected WT mice. In this same sense, we detected similar changes in the presence of CoPEC in APC mutant-status RCC patients' tumors and APC-mutated HT-29 cells.

Conclusion: This work clarifies how CoPEC may shape tumor heterogeneity through their influence on lipid metabolism and will allow exploration into the mechanisms of CoPEC-mediated lipid reprogramming on the efficacy of antitumoral therapy.

Keywords : Colorectal cancer, Colibactin and lipids

P39 - The mTOR substrates 4EBP1/2 reprogram fatty acid metabolism to promote survival under energetic stress

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Solid tumor cells are developing within hostile microenvironments characterized by oxygen and glucose deprivation, due to the defective nature of tumor vasculature. While nutrient-deprived conditions primarily restrict tumor growth, it may adversely promote the emergence of more highly aggressive tumor clones through selective pressure. We hypothesized that in order to adapt to energetic stress, tumor cells exploit conserved signaling pathways mediating the physiological response to energetic stress, such as the nutrient-responsive mTOR pathway. Here, we found that the mTOR substrates and mRNA translation regulators Eukaryotic translation initiation factor 4E-binding protein 1/2 (4EBP1/2) are required for cell survival under glucose deprivation in multiple cellular models. This function is highly conserved in the evolution as we uncovered that the yeast ortholog of 4EBP promotes viability of S. cerevisiae under glucose deprivation. We characterized that the pro-survival function of 4EBP1/2 relies on the control of NADPH homeostasis to prevent oxidative stress when glucose is scarce. Mechanistically, 4EBP1/2 selectively block the translation of acetyl-CoA carboxylase 1 (ACC1) under glucose starvation in turn inhibiting fatty acid synthesis, one of the most highly NADPH consuming process in a cell. Furthermore, we highlighted that by controlling ACC1 expression 4EBP1 promotes oncogenic transformation and glioma tumorigenesis in vitro and in vivo, in contrast to the widely accepted tumor suppressive function of 4EBP1. This has clinical relevance as we observed that 4EBP1 is overexpressed and is a factor of poor prognosis in numerous human cancers. Altogether, our work reveals that the mTOR substrate 4EBP1 confers cellular protection under glucose-deprived conditions, which is hijacked by glioma cells to support their tumorigenicity.

Keywords : Energetic stress, mTOR, fatty acids

P40 - Metabolic bases of cell plasticity: LKB1-p53 crosstalk in neural crest stem cells and lung adenocarcinomas

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Cells constantly adjust their metabolism to their environment through signaling pathways that act as biosensors of nutrient availability, such as LKB1 signaling. This tumor suppressor and master kinase regulates 14 downstream kinases among them AMPK, a metabolic sensor of ATP/ADP ratio. Under metabolic stress, AMPK restores energy of the cell by activating catabolic reactions and inhibiting anabolic processes, notably the mTOR pathway. LKB1 signaling is thus essential to adapt cell fate to nutrient availability through the control of various cellular processes such as stem cell quiescence, proliferation or differentiation1. Our group is exploring the metabolic regulations exerted by LKB1 signaling in several developmental and tumorigenic contexts.

Based on data from the literature, we hypothesized that metabolic regulations exerted by LKB1/AMPK could contribute to neural crest cell formation, a fascinating stem cell population contributing to the shaping and function of many organs. To assess this hypothesis, we generated genetically engineered mouse models of spatio-temporal inactivation of Lkb1 in neural crest cells2,3. In-deep phenotyping using histological approaches combined with 3D morphometric analyses established that LKB1 is a new master regulator of various NCC lineages. In the glial lineage, which gives rise notably to Schwann cells of the peripheral nerves, we uncovered that LKB1/AMPK governs cell behavior by connecting mTOR activity to pyruvate-alanine cycling and glutamate-glutamine conversion. We also highlighted that LKB1 prevents oxidative DNA damages and p53 activation, a necessary process to drive healthy cell fate both in vitro and in vivo4. Altogether, these data further suggest that disruption of the LKB1 signaling contributes to the pathogenesis of neural crest diseases5.

Neural crest cells share many properties with cancer cells, such as metabolic rewiring, delamination by an epithelial-to-mesenchymal transition, long distance migration...6,7 We therefore also examined how LKB1-metabolic regulations identified in neural crest cells contribute to LKB1 tumor suppression activity in lung adenocarcinomas (LUAD). We uncover that loss of LKB1 function in LUAD is associated with massive downregulation of transcription factors. Besides, we showed that nuclear LKB1 interacts with the chromatin remodeler BRG1 and the transcription factor p53 and its loss associates with increased chromatin-based repression. Consequent to wide transcriptional and epigenetic deregulations, LUAD patients exhibited a downregulated neuronal-glial signature, an increased oxidative stress and impaired amino acid homeostasis. This analysis therefore identified that LKB1 functions during both development and tumor suppression share molecular similarities, reinforcing the relationship between developmental and tumorigenic processes8.
As an emerging project in our group, we now explore how LKB1 activity in nerve cells could impact lung adenocarcinoma cell colonization into nerve fibers at proximity of the tumor, a process named perineural invasion (see abstract by Jordan Allard).

Collectivelly, our research thus aims at characterizing the molecular mechanisms -notably metabolic regulations- that underlie LKB1 functions in the control of cell plasticity in physiopathological conditions.

Keywords : LKB1 signaling, energy metabolism, cell fate, neural crest cell, lung adenocarcinoma

P41 - Extracellular matrix remodeling favors lineage specific transformation in the lung

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One of the major difficulties in the management of lung cancers is their great heterogeneity of response to therapeutic treatments, due to the difference in cellular origin. Three progenitor cells can be at the origin of lung adenocarcinoma: the alveolar type 2 cells (AEC2) located in the pulmonary alveolus, the club cells located in the bronchiol and the bronchoalveolar stem cells (BASC) located at the bronchoalveolar junction. A better understanding of the factors influencing the tumor transformation of these cells would allow the development of new therapies. The maintenance and activation of stem and progenitor cells is finely regulated by their interaction with their microenvironment (niche) which is constituted by the extracellular matrix (ECM). ECM rigidity plays a crucial role in stem cell fate. A modulation of the ECM properties, such as stiffness, can lead to a deregulation of the stem cells impacting their proliferation and differentiation capacity. The differential localization of progenitor cells implies an interaction of these cells with a specific ECM in term of composition and mechanical properties. Tumor initiation being influenced by multiple genetic and environmental factors, we wondered whether ECM remodelling and stiffness sensing could influence tumor cell of origin transformation. SLC3A2, a transmembrane protein highly expressed in lung cancer, is a sensor of the microenvironment involved in ECM remodeling via its interaction with beta integrins. To determine the impact of SLC3A2 loss on tumor initiation, we used a well characterized mouse model (KrasLSL-G12D/+; Trp53fl/fl) that recapitulates the full cascade of lung adenocarcinoma development. Tumor-initiating cells infected with adenoviral Cre undergo hyperplasia, progression to adenomas, and finally to adenocarcinomas. We found that while SLC3A2 is expressed in alveolar and in bronchiolar tumors, loss of SLC3A2 promotes a tumor switch from bronchiolar to alveolar origin. It also decreases tumor collagen assembly suggesting a softening of the tumor ECM. Using adenoviruses in which Cre expression is driven by progenitor promoters, targeting either AEC2 (using Sftpc surfactant-associated protein

C, SPC), or club cells (using CC10 uteroglobulin SCGB1A1), we found that loss of SLC3A2 specifically impairs club-cell malignant transformation and tumorginesis. Consistent with these results, SLC3A2 is enriched in a sub-population of progenitor cells within the bronchiol in homeostasis. Together, our results suggest that stiffness sensing is lineage-specific and can lead to transformation, pointing toward a critical role of cell-of-origin in adenocarcinoma heterogeneity.

Keywords : Lung cancer, Tumor cell of origin, Stem/progenitor cells, ECM remodeling

P42 - 2-Oxoglutarate regulates tumor invasiveness through co-activated pathways that converge on Snail

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Metabolic reprogramming, a recognized hallmark of cancer, plays a critical role in tumor invasion and metastasis. Recent findings revealed that deficiencies in metabolic enzymes can lead to altered cellular 2-oxoglutarate (2-OG) levels, suggesting a possible function for 2-OG as a key metabolite in tumor progression. Besides its metabolic role as a TCA cycle intermediate, 2-OG serves as a co-substrate of 2-OG-dependent dioxygenases (2-OGDDs) which catalyze critical biological processes linked to cancer progression, including hypoxia sensing, epigenetic modification, and extracellular matrix remodeling. Additionally, 2-OG directly inhibits ATP synthase resulting in reduced cellular ATP levels and diminished mTOR activity in various experimental models. However, it remains to be elucidated how changes in 2-OG homeostasis impact tumor cell invasiveness, in which epithelial-to-mesenchymal transition (EMT) is a key event. To determine the role of 2-OG on the regulation of EMT and invasion, we modulated 2-OG levels by either supplementing the tumor cells with a cell-permeable form of 2-OG or depleting 2-OG levels through the knockdown of isocitrate dehydrogenase 1 (IDH1). Several functional assays such as Boyden chamber, western blot, immunofluorescence, RT-qPCR, and metabolite mass-spectrometry analyses were employed to explore the underlying mechanisms. Strikingly, 2-OG supplementation reverts TGFβ-induced invasion and among the master regulators of EMT (Snail, Slug, ZEB1, ZEB2, Twist1, and Twist2), we observed the most prominent changes in Snail, both at RNA and protein levels. Moreover, IDH1 knockdown enhanced tumor invasion and metastasis. Importantly, we uncovered two molecular mechanisms through which 2-OG-driven Snail regulation is mediated; PHD/HIF-α and mTOR/c-Myc signalling. Collectively, our results highlight the interplay between metabolism and metastasis and reveal novel mechanistic insights into the impact of altered 2-OG homeostasis.

Keywords : 2-oxoglutarate, metabolism, EMT, Snail, metastasis

P43 - Development and characterisation of a novel 3D in vitro model of obesity associated breast cancer as a tool for drug testing

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Obesity is associated with increased breast cancer aggressiveness and decreased chemotherapy efficacy. However, the underlying mechanisms of how obesity mediates chemotherapy resistance remains unclear. This may in part be due to a lack of suitable models capable of recapitulating the tumour microenvironment associated with metabolic dysregulation in obesity. Therefore, our aim was to develop a 3D in vitro model of obesity-associated breast cancer, as a tool for drug testing.

To generate a novel model of obesity-associated breast cancer, hypertrophic adipocyte spheroids were generated and co-cultured with breast tumour cells, myoepithelial cells, macrophages, and fibroblasts in a collagen type I matrix. Adipocytes were treated with palmitate for 12 days to mimic caloric overload and induce a hypertrophic phenotype, characterised by increased lipid droplet size and downregulation of PPARG, and FABP4 mRNA levels.

Organotypic cultures containing adipocytes showed increased proliferation and stemness of breast tumour cells. Infiltration of breast tu-

mour cells and macrophages was observed within and surrounding adipocyte spheroids, replicating the adipose-inflamed border observed in obese breast cancer patients. Immunohistochemical staining of organotypic cultures revealed that macrophages co-localise with adipocytes, forming crown-like structures. Interestingly, macrophages surrounding adipocytes exhibited both CD32b and CD16 expression, suggesting a metabolically dysregulated phenotype. This organotypic system was then utilised as a drug testing platform. Organotypics were treated with Paclitaxel, Doxorubicin, Tamoxifen or Metformin for 48 hours and assessed for cell viability via CellTiter-Glo® assay. Cultures containing hypertrophic adipocytes exhibited increased sensitivity to Metformin and resistance to Paclitaxel, compared to lean cultures. We are currently performing NMR-based metabolomics to identify further metabolic vulnerabilities for drug testing.

This study proposes a 3D organotypic model which recapitulates the obese adipose environment in breast cancer patients. This model provides a useful tool to interrogate the metabolic crosstalk between adipocytes and breast tumour cells and to investigate mechanisms underpinning obesity-related chemoresistance.

Keywords : Obesity, Breast cancer, Tumour microenvironment, Drug resistance

P44 - The metabolism of breast cancer to the lung: Key pathways and their implications in the metastatic microenvironment

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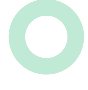
Introduction
Metastasis is still responsible for the majority of cancer-related deaths. Metabolic adaptation is emerging as a hallmark of metastasis, and targeting its specific metabolic features is an interesting and unexplored therapeutic window (1). We focus on the oncogene c-MYC, which drives metabolic reprogramming, and it is associated with high outgrowth capacity and poor prognosis (2). Our model of study is Myc-driven mammary gland tumour mouse model, MMTV-Myc, together with a panel of cell lines with enriched metastatic capacities, derived from this model. With this combined in vivo and ex vivo model, our aim is to explore the key metabolic pathways that drive metastatic colonization from breast cancer to the lung, and their implications in the tumour microenvironment.

Materials and methods
We use conventional stable-isotope resolved metabolomics techniques supported by GC/MS and LC/MS, that allow us to determine activities of certain pathways that are still unexplored in the context of metastasis. Together with this, we use cutting-edge technologies such as mass spectrometry imaging, imaging cytometry and single-cell RNA sequencing, to deeply understand the metabolic interactions between the tumour and its microenvironment.

Results
According to our initial experiments, the metabolic features of breast cancer metastasis to the lungs include the upregulation one carbon-metabolism and antioxidant pathways, which is also in agreement with recent publications (3,4). Metastatic tumours encounter a high availability of methionine cycle intermediates in the lung, and present increased levels of glutathione. Furthermore, we have determined amino acid vulnerabilities that are specific for the metastatic-enriched subpopulations, as well as a higher pathway activity of glutathione production, that might be essential for the success in metastatic outgrowth.

Conclusion
Overall, the implications of this work are the discovery of new metabolic mechanisms of metastasis that eventually contribute to the design of more efficient therapeutic approaches for breast metastatic cancer.



P45 - Lactic acid primes prostate cancer cells to undergo a quiescent state

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Cancer cells initiate adaptation mechanisms to adapt tightly regulated cellular processes to non-optimal growth environments such as metastatic niches. However, primary tumours are heterogeneous in composition, possibly influenced by the microenvironment, and this may dictate phenotypic differences in the localized tumor populations, including a distinct long-term post-dissemination behavior. Breast and prostate cancers (PCa) are tumours able to progress rapidly, but also to become quiescent accordingly to the environmental stimuli. Metabolic environment of the primary tumor has been recognized as a key determinant in the progression of most solid tumours. Lactic acid (LA) is one of the most abundant metabolites in both circulation and primary tumours, thereby sustaining the aggressiveness of tumor cells, including PCa cells. However, how LA influences the fate of disseminated tumour cells in target organs is still unclear.
We treated PCa cell lines (PC3, DU-145) with 20mM LA, a concentration found in most solid tumors.
RNA-seq analysis of PCa cells exposed to LA revealed a peculiar gene program (upregulation of extracellular matrix and invasiveness gene pathways, paralleled to downregulation of cell cycle), which correlates with a quiescent cell signature. We found that LA administration in vitro delays PCa cells proliferation after 5 days. Also, LA-exposed PCa cells exhibited the expression of dormancy markers such as NR2F1 and p27, as well as a decreased levels of proliferative markers such as p-ERK and MERTK. Venus-p27K cell cycle probe showed an increased proportion of G0-quiescent PCa cells induced by LA, suggesting that LA may control the arise of the quiescent populations in PCa. Also, by using a lipophilic fluorescent dye, DiD, to monitor proliferative and non-proliferative cells, we found that DiD+ cells exhibit a higher lipid droplets content, upon exposure to LA, suggesting that lipid metabolism may be important in maintaining the quiescent state of the cells.
Our preliminary data suggest that LA is crucial for selecting invasive, quiescent tumour cells able to sleep for a long or to reawake accordingly to the external environment.

Keywords : quiescence, metastasis, prostate cancer, lactic acid

P46 - MULTIPLE MYELOMA GLUTAMINE METABOLISM SHAPES A PRO-TUMOUR BONE MARROW NICHE

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Derangement in cancer metabolism not only sustains neoplastic cell growth, but also may impact on other cell population of the tumor microenvironment. Multiple myeloma (MM) is a proliferation of malignant plasma cells in the bone marrow (BM), characterized by bone lesions and increased adiposity. We have already demonstrated that the glutamine addiction of MM creates a low-glutamine/high glutamate metabolic microenvironment, a phenomenon that sustains Glutamine Synthetase (GS) expression and impairs osteoblast (OB) differentiation of mesenchymal stromal cells (MSCs), thus favoring bone destruction. However, no study on the metabolic interaction among MM cells and MSCs or adipocytes are available and are investigated in this study.

Primary human BM MSCs from healthy donors and human MM cell lines were grown in RPMI1640 medium supplemented with 4mM glutamine and 10% FBS. MSCs were incubated in either adipogenic (0.5 mM 3-Isobutyl-1-methylxanthine, 5μM indomethacin, 50μM dexamethasone and 10mg/ml human insulin) or osteogenic medium (10-8 M dexamethasone and 50 μg/ml ascorbic acid) medium for 14-days. Glutamine secretion was measured by mass spectrometry. 3H-Glu was used to determine EAAT3 activity. Cell viability was evaluated by the resazurin assay.
13C metabolite tracing reveals that in MM cells more than 50% of glutamate directly derive from glutamine deamidation.
However, MM cells discard high amount of glutamine-derived glutamate in the extracellular space through SLC7A11 transporter, whose expression increases during MM malignant progression. On the other hand, undifferentiated MSCs, but not OBs, display sodium-dependent glutamate uptake and higher expression of the EAAT3 sodium-dependent glutamate transporter. Consistently, public transcriptional profiles of BM biopsies of healthy donors (n=7) or MM patients (n=16) revealed that the expression of the inward glutamate transporter EAAT3 is higher in MSCs compared to OBs. In glutamine-free conditions, MSCs produced and secreted higher amount of glutamine than OBs. In MSCs, but not in OBs, glutamine secretion is further boosted by extracellular glutamate supplementation, while is hindered by either GS or

EAAT3 inhibitors. In co-cultures, MSCs support MM cell viability under glutamine shortage, while this nutritional support is markedly hindered by either the inhibition or the silencing of GS or EAAT3 in MSCs, with a substantial decrease in MM cell viability. Thus, MSCs recycle MM-secreted glutamate to synthetize glutamine, thus supporting malignant cell growth thanks to the activity of EAAT3 and GS. Lastly, in MSCs incubated under adipogenic conditions, glutamine deprivation increased lipogenesis, assessed with Oil Red O staining, and the expression of the adipocyte markers PPARG, LEP and ADIPOQ.

These data point to a MM-driven metabolic pro-tumor BM niche in which glutamate secretion from MM cells is functional to sustain their own glutamine addiction thanks to the ability of MSCs to exploit MM-derived glutamate to synthetize and secrete glutamine. Moreover, MSC differentiation is skewed from osteogenesis to adipogenesis even if the effects on the oversized adipocyte population described in MM, remain to be characterized. In conclusion, several steps of these deranged pathways are sensitive to pharmacological inhibition and may constitute novel therapeutic approaches to counteract MM growth or its effects on the BM niche.

Keywords : Multiple Myeloma, Glutamine Metabolism, Glutamate Transport, Mesenchimal Stromal Cells, Adipocyte Differentiation

P47 - Breast cancer cells rely on mitochondrial respiration to form bone metastases

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Bone metastases formed by breast cancer cells cause severe morbidity. Current therapies reduce the pain and inhibit further progression, but they do not cure, highlighting the need for novel therapeutic strategies.
The metabolic flexibility of cancer cells controls their metastatic ability, but knowledge on the metabolic profile that regulates bone metastasis formation is still limited. We therefore performed scRNA-sequencing analysis on the early stages of bone metastases formed by mouse 4T1 breast cancer cells and compared the transcriptome to the one of primary tumors. The gene expression profile showed that breast cancer cells at the mammary site are highly glycolytic, but switch to oxidative phosphorylation (OXPHOS) when they metastasize to the bone. We therefore questioned whether we could reduce the formation of bone metastases by inhibiting OXPHOS.
To this end, we silenced NDUFV1, an essential component of Complex I of the electron transport chain (NDUFV1KD), using a doxycycline-inducible CRISPR/Cas9 system, which resulted in decreased complex I activity and oxygen consumption rate, as assessed by Seahorse analysis. Orthotopic implantation of NDUFV1KD cells in immunodeficient female mice resulted in smaller mammary tumors with a 40% reduction in tumor weight. Upon intracardiac injection, NDUFV1KD cells formed significantly fewer bone metastases, with only 4/11 mice showing bone lesions, whereas all mice (9/9) injected with control 4T1scr cells developed bone metastases, as analyzed by microCT. In addition, histological analysis showed that metastatic tumor burden in bone was manifestly lower (-90%) after injection of NDUFV1KD cells compared to 4T1scr cells, indicating that breast tumor cells require OXPHOS to form bone metastasis. Accordingly, mice injected with 4T1scr cells lost a substantial amount of bone (-33%), whereas bone mass was preserved in mice inoculated with NDUFV1KD cells (-1.5%). Similar in vivo results were obtained upon silencing a Complex II essential component (SDHA). Bone metastatic tumor burden was reduced by 85% while mammary tumor weight was only reduced by 40%. These data indicate that 4T1 cells are more dependent on OXPHOS to form bone metastases than mammary tumors. Mechanistically, we observed a decreased cell proliferation in vitro in NDUFV1KD cells. Metabolically, NDUFV1KD cells became more glycolytic, but this adaptation could not prevent that NDUFV1KD cells were in energy stress, evidenced by increased pAMPKα/AMPKα ratio. In addition, ROS levels were increased, despite higher glutathione levels, although this oxidative stress did not result in cell death. Additionally, nucleotide and fatty acid synthesis intermediates were decreased (LC-MS: -50% aspartate, -90% malonyl-CoA), and this impaired biosynthesis likely contributed to the growth defect.
Taken together, we show that OXPHOS is crucial for breast cancer cells to form bone metastases by supporting biosynthesis and bioenergetics. Our data also highlight that breast cancer cells forming bone metastases require specific metabolic adaptations.

Keywords : Oxidative phosphorylation, Breast Cancer, Bone Metastasis

P48 - Despite a regular spontaneous physical activity, a long-term high-fat diet promotes mammary cancer development in mice.

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Breast cancer is the most women common malignancies worldwide(1). Among multiple risk factors, sedentary lifestyle, obesity and post-menopausal are correlated to estrogen and inflammation exposure during a lifetime(2). Physical activity protects against breast cancer development by affecting hormone levels, immune responses, and oxidative defences(3,4). The impact of long-term obesity on the physical activity (PA) benefits in preventing and managing mammary tumorigenesis was studied.

Ovariectomized 35-week-old C57BL/6 mice were placed in an enriched environment to induce spontaneous PA and fed throughout the experiment with a high-fat diet (HFD). After 42 days (Short-term(ST), n=10) or 88 days (Long-term(LT) n=10) of exposure to HFD, syngenic mammary EO771 cells were implanted into the 4th mammary glands and the tumour growth was followed for 30 days. At sacrifice, the tumour microenvironment (TME) immune infiltrate and metabolic parameters were explored using flux cytometry, transcriptomic, enzyme activities and biochemical approaches. The data reported as mean±SD were analysed by a Mann-Whitney test.
The median survival was significantly reduced in the LT group compared to the ST group (22 days vs 25.5 days, p=0.0296). Spontaneous PA and food intake were similar before (0.645±0.082 vs 1.065±0.076km/mice/j; 2.786±0.154 vs 2.835±0.066g/d) and after tumoral implantation (0.0803±0.045 vs 0.965±0.217 km/mice/d; 2.264±0.208 vs 2.713±0.081g/d). At sacrifice, the visceral adipose tissue mass was higher in the LT group (1029.88±203.77 vs 1533.40±259.79mg, p=0.04) while the skeletal muscle mass was reduced (354.20±12.79 vs 334.14±7.83mg (ST group), p=0.0765). In the TME, among the total lymphocytes, the proportion of NK cells was reduced in the LT group (24.54±1.93 vs 0.24±0.09%, p=0.05) as well as the TCD8+ one, (7.87±3.07 vs 1.01±0.28%, p=0.002) while the proportion of T regulators tended to increase (0.02±0.01 vs 0.50±0.06%, p=0.1), leading to a collapse of the T8/Treg ratio (421.61±153.84 vs 2.62±1.31, p=0.03). The significant decrease in the tumour triglyceride content in the LT group (0.07±0.01 vs 0.01±0.01mmol/g of tissue, p=0.0143) was accompanied with an enhanced activity of the glutathione reductase (165.46±5.81 vs 224.83±26.80UI/mg of proteins, p=0.0286) and a decrease of the glutathione S transferase activity (295.40±15.10 vs 11.61±1.79UI/mg of proteins, p=0.0143), markers of glutahione recycling involved in the oxidative stress management.

In our experimental conditions, the LT HFD is associated with the tumor growth despite the spontaneous PA and promotes a tolerogenic TME by increasing lipid consumption and oxidative stress and recruiting anti-tumour immune cells. The tumor and skeletal muscles exploration by the analysis of cytokines, myokines and free radicals could help to understand the inter-organ exchanges related to tumor development and muscle mass loss. In perspective, the combination of imposed PA with immunotherapy treatment could be envisaged to counteract the long-term effects of a hypercaloric diet.

Keywords : High-fat diet, Physical activity, Breast tumor microenvironment, Immunity,

P49 - ALTERED MITOCHONDRIAL STRUCTURE REVEALED BY SUPER-RESOLUTION IMAGING AFTER TREATMENT WITH THE NOVEL HISTONE DEACETYLASE 6 (HDAC6) INHIBITOR, BAS2

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Triple-negative breast cancer (TNBC) mainstay of treatment is non-selective cytotoxic chemotherapy. Unfortunately, for approximately 50% of patients, the disease will relapse and recur. To identify compounds that kill apoptotic-resistant TNBC cells, but spare normal breast, our lab conducted a small-molecule screen. A novel histone deacetylase 6 (HDAC6) inhibitor, BAS-2, was identified that regulated glycolytic metabolism.
Fumarate hydratase (FH) is an enzyme present in the mitochondrial matrix where it participates in the metabolic pathway, the citric acid (TCA) cycle. FH converts fumarate to malate and dysregulation has been linked to cancers, such as hereditary leiomyomatosis and renal

cell cancer. Herein, we uncover an interaction of HDAC6 with FH, altered FH activity, and distinct mitochondrial aberrations after HDAC6 inhibition.

Cell metabolism was investigated using 13C6-glucose mass-spectrometry (MS). FH activity was determined by enzymatic assay and immunoprecipitation (IP) via antibody pull-down. Annexin V flow cytometry was used for cell death. Mitochondria were examined by transmission electron microscopy (TEM) and live-cell stimulated emission depletion (STED) imaging.

The HDAC6 inhibitor, BAS2, was first confirmed to reduce viability of MDA-MB-231 and BT549 TNBC cell lines. Interestingly, it was identified by C6 labelling that HDAC6 inhibition caused increased fumarate, and a reduction in FH activity was confirmed by enzymatic assays. The interactome of HDAC6 revealed binding with FH, that was confirmed by HDAC6 IP and validated by confocal imaging. Interestingly, exogenous fumarate also induced apoptosis, suggesting a detrimental effect of fumarate accumulation for TNBC.
Recently, FH loss and resultant fumarate accumulation have been associated with mitochondrial cristae and mtDNA alterations. We investigated this using state-of-the-art live-cell super-resolution nanoscopy. Excitingly, we observed stark cristae aberrations in live-cells following inhibition of HDAC6, which were confirmed by TEM. This was associated with expansion of mtDNA and appearance of mtDNA in the cytosol, a known stress signal.

Here we report a novel function for HDAC6 in the regulation of mitochondria structure, function, and metabolism. HDAC6 has been found to regulate glycolysis, and now to affect mitochondrial structure. Importantly, these findings point to a unique metabolic vulnerability in TNBC cells through regulation of metabolism by HDAC6.

Keywords : HDAC6, triple negative breast cancer, mitochondrial metabolism, super-resolution

P50 - Dietary Lipid, Palmitate, Alter Critical Metabolic Reprograming Genes in Meningioma

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In our study, we explore the connection between poor dietary habits, specifically high sugar and fat intake, and cancer, focusing on meningiomas, the most common primary adult brain tumor. Our project examines the impact of palmitate, a common dietary lipid, on the metabolic reprogramming of meningiomas. It's known that abnormal fatty acid (FA) metabolism is linked to worse prognosis in meningiomas, but the underlying molecular mechanisms are not well understood (1).
In our model, we evaluated meningioma cell lines, CH157-MN, IOMM Lee and AC599, reflecting each grade of meningioma disease. Using the MTT viability assay, we determined the tolerable palmitate ranges for each cell line and found out that these ranges are comparable across all grades. Consequently, keeping extracellular levels of palmitate as our constant variable, we mimicked a fat rich extracellular tumor micro environment, with the aim to investigate the gene expression changes caused by palmitate exposure. To uncover critical reprogramming genes that are responsive to elevated microenvironmental palmitate levels, we processed our RNAseq data from the library we have built for our cell lines using a robust, carefully curated database tool; Ingenuity Pathway Analysis (IPA). Then, via QPCR we determined how the expression profiles of our candidate genes change upon palmitate treatment.
Our analysis revealed the downregulation of the fatty acid metabolism enzyme Aldehyde Oxidase 1 (AOX1). The loss of AOX1, increasing NADP levels, which in turn boost carbon flux through the pentose phosphate pathway (PPP), nucleotide synthesis, and cell invasion, has been implicated in metabolic deregulation of prostate cancer as well (2). Similarly, our data suggests AOX1 involvement in aberrant FA metabolism of meningiomas. Notably, AOX1 downregulation was connected to a higher tumor grade, implying that it may be an adaptation that malignant meningioma cells use to improve their potential to survive and form tumors.
So far, our findings suggest that targeting the fatty acid axis could be a promising therapeutic method for treating malignant meningiomas, offering insight on a yet-to-be discovered element of their metabolic adaptability. In future investigations, we intend to use cutting-edge genetic manipulation tools to better understand the biological function of AOX1 downregulation and how it benefits growing tumors

Keywords : Tumour microenvironment, Meningioma, Palmitate, AOX1

P52 - Tumor metabolic reprogramming under L-asparaginase treatment

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Introduction: Diffuse B Large Cell Lymphoma (DLBCL) is the most common and aggressive type of Non-Hodgkin's B-cell lymphomas. Since more than two decades, patients with DLBCL are treated with an immuno-chemotherapy (R-CHOP), which significantly improved their overall survival. However, 40% of patients still experience therapeutic failure. We recently established the proof of concept of L-asparaginase (ASNase) anti-tumor efficacy in patients with R-CHOP-refractory DLBCL. ASNase is an enzyme catalyzing the hydrolysis of L-asparagine in the bloodstream and is clinically used to treat patients with acute lymphoblastic leukemia. Patients with R-CHOP refractory-DLBCL treated with ASNase had a regression of their tumor burden (1), but all patients eventually relapsed, suggesting the presence of remaining cancer cells capable of adaptation to ASNase treatment.

Objectives: Mechanisms involved in tumor cells' adaptation to ASNase treatment are unexplored. Using a preclinical model of murine B-cell lymphomas, we set up in vitro and in vivo models to address this issue.

Methods and results: By metabolomic analysis, we identified metabolic deregulations in malignant B cells treated with ASNase in vitro and in vivo. The metabolic flexibility of malignant B cells surviving ASNase treatment is characterized by an increased intracellular L-serine levels, a feature described to favor tumor progression. Increased total intracellular L-serine levels is independent of serine uptake but it is associated with an increased expression of enzymes involved in its de novo biosynthesis (PHGDH, PSAT1 and PSPH). Using 13C6-Glucose and 15N(amine)-L-Glutamine, we demonstrated an increased levels of newly synthetized serine by malignant B cells surviving ASNase treatment in vitro and in vivo. Moreover, combining ASNase with a PHGDH inhibitor (BI4916) increased cell death and delayed tumor cell's adaptation to ASNase treatment in vitro.

Conclusion: Our results together suggest the role of serine metabolism in escape mechanism of asparagine-restricted cancer cells.

Keywords : B-cell lymphomas, L-asparaginase, Metabolic adaptation, Therapy

P53 - Nutrient-sensitized CRISPR/Cas9 screening reveals novel players in pyrimidine biosynthesis

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Introduction
Rapidly proliferating cells, such as cancer cells or active immune cells, require de novo nucleotide synthesis to support DNA replication and growth. The inhibition of these pathways represents a potent therapeutic strategy in oncology and beyond, but our incomplete understanding of synthesis limits target discovery.

De novo pyrimidine synthesis consists of only three enzymes, and inhibition of these is currently used in clinics or in clinical trials to treat viral infection, cancers, or inflammatory autoimmune disorders, with encouraging results. In contrast, the regulation of these three enzymes by other factors, such as proteins and metabolites, is less understood, but holds important therapeutic potential.

Here, we designed a nutrient-sensitized screen to identify new players in pyrimidine biosynthesis at the genome scale.

Methods
We used genome-wide CRISPR/Cas9 screening and compared the growth of K562 cells in the presence or absence of uridine, a nucleoside that can be salvaged when de novo synthesis is not possible. We followed up on this screen with individual gene knock-out and metabolomics to further characterize the effect of genes of interest on each step of pyrimidine synthesis.

Results
Confirming our approach, we identified as top hits the three main enzymes of pyrimidine synthesis. Those genes were followed by biosynthetic enzymes for coenzyme Q, a known cofactor in pyrimidine synthesis.

Importantly, our screen also highlighted multiple genes not previously linked to nucleotide synthesis, some of which have established roles in cancer. Using targeted metabolomics, we found that these represent novel regulators of each of the three main steps of pyrimidine de novo synthesis.

Conclusion
Through the identification of novel players in pyrimidine de novo biosynthesis, we expand our understanding of this pathway. We are currently investigating the mechanism by which these genes regulate nucleotide metabolism and their therapeutic potential.

Keywords : Nucleotides, Genetic screening, Metabolomics, Cancer

P54 - Contextualization of Metabolic Network Models and their Application to Drug Repurposing

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Introduction
Mathematical modelling of metabolic networks is a powerful tool to study metabolism on a genome-scale level. It allows to discover the activity of pathways of interests, the amount of produced or exchanged metabolites, and to elucidate essential and specific metabolic genes amongst many other applications. The most widely employed constraint-based modelling approach uses linear programming on mass balance equations in steady state further enriched with thermodynamical constraints. Often the optimization of a reasonable objective function (like growth or ATP maintenance) is pursued thereby.

Material and methods
To increase the predictive power of such metabolic models, we develop fast and powerful large-scale data integration methods which enable the reconstruction of context-specific molecular networks, e.g., for a given disease, patient group or individual patient. The fastcore family of algorithms allows the integration of large-scale gene expression data and other data types with generic metabolic reconstructions for producing specific molecular metabolic networks [1]. This is powered by an efficient linear programming approach, which allows to obtain a close-to-optimal minimal network given a core set of metabolic reactions. Largely applied also by other teams, these algorithms have been included in a community-effort toolbox [2]. This has lately been extended with a novel dynamic Flux Balance Analysis approach for multi-tissue metabolic modelling and allows now also for simulating disease-specific metabolic blood level alterations [5]. And a recently released single cell version of the algorithm allows to study the metabolic activity of the different cell types captured with single cell-RNAseq data, as well as their putative metabolic crosstalk in terms of exchanged metabolites [4].

Results
Based on these and other state-of-the-art computational biology, data science and machine learning approaches, we developed a variety of fruitful collaborations, notably in cancer research. E.g., we employ the reconstructed cancer specific molecular networks for identifying promising specific targets and to suggest novel treatment strategies. Drug repurposing thereby aims at reorienting approved drugs to novel disease indications. In proof-of-concept studies we used fastcore to predict several non-cancer drugs to be effective in colorectal cancer [1], melanoma [3], or glioblastoma [under preparation], while less harming healthy control tissue. The experimental validation gave superior results compared to large-scale screening efforts. We could also show that synergistic effects of such metabolic drugs in combination with state-of-the-art targeted signalling drugs are possible [3].

Conclusion
In summary, large scale metabolic modelling combined with powerful data integration methods is an excellent tool to enhance the discovery of novel metabolites and pathways of interest, as well as of new drug candidates.

Keywords : Metabolic networks, Constraint-based modelling; Network analysis; Cancer; Drug repurposing

P55 - Cytotoxicity-related sphingolipidomic changes induced in double-hit Diffuse Large B-Cell Lymphoma after treatment with statins in vitro

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Diffuse Large B-Cell Lymphoma (DLBCL) is the most common hematologic malignancy in the world and one of the deadliest. Since the introduction of anti-CD20 blocking antibodies, prognosis has dramatically improved; however, over 40% of cases are resistant or recur after an initial remission. These outcomes largely depend on its underlying genomics, which makes it difficult to approach because it is also one of the most genetically diverse neoplasms. Therefore, research beyond its already extensively described molecular biology is necessary. Metabolic reprogramming at the lipid level is one of the least understood hallmarks of cancer, but its relevance is becoming increasingly evident because it accurately depicts cellular phenotype and because of the bioactive nature of many lipid species such as sphingosine and ceramide.

Statins are widely prescribed drugs. Although they are classically used as cholesterol lowering medications, they are incredibly pleiotropic agents and are known to have antilymphomagenic properties. However, the underlying mechanisms leading to these effects are still unclear. In this work, it was hypothesized that statins induce a metabolic shift that promotes the accumulation of cytotoxic lipids, triggering cell death. To approach this, an aggressive lymphoma cell line (RC) was treated with different combinations of statin-containing therapeutic regimens. High-potency statins with different physicochemical properties were used (atorvastatin and rosuvastatin) as either single agents or in combination with the classic R-CHOP therapy. Cell cycle was assessed through flow cytometry, and a method for sphingolipid determination was developed for mass spectrometry.

We observed that the combination of R-CHOP + either statin significantly increased the proportion of cells in G0/G1 as compared to R-CHOP alone or atorvastatin alone. Additionally, R-CHOP + atorvastatin decreased the proportion of cells in S and G2/M phase as compared to either treatment individually. These findings suggests that R-CHOP + atorvastatin is particularly efficient at inducing G1/S cell cycle arrest. Furthermore, this same combination also increased sphingosine and sphinganine levels as compared to atorvastatin-only group. Unexpectedly C16 ceramide levels fell upon treatment with any regimen.

Our data shows that statins have cytotoxic properties against double-hit lymphoma in vitro, and that lipophilic statins (atorvastatin) might have a more pronounced effect. Furthermore, our results suggest that atorvastatin synergistically induces G1/S cell cycle arrest with R-CHOP immunochemotherapy. This is an interesting observation because most components of the R-CHOP regimen are either cell-cycle independent or impact predominantly the G2/M phase, suggesting that this shift is specifically related to the presence of atorvastatin. The exact mechanisms of statin-related cytotoxicity are unclear and likely multidimensional: cholesterol deprivation, lipid raft disruption, calcium signaling dysregulation, and deficient isoprenylation to name a few. Here, we show that a metabolic shift in sphingolipid metabolism might be partially responsible for this effect.

To the best of our knowledge, this is the first work to evaluate the sphingolipidomic effect of statins in the cancer context and gives rise to clinical research that could lead to novel biomarkers and drug repurposing.

P56 - Interplay of glycolysis and gluconeogenesis in lung cancer cells

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Introduction
Lung cancers consume large amounts of glucose. However, as shown in recent tracing experiments in humans, they display metabolic intra- and intertumoral heterogeneity and use different carbon sources. Partly, this may be due to a regional shortage of important nutrients such as glucose. It has been shown by us and others, that lung cancer cells can use initial steps of gluconeogenesis to fuel glycolysis branching pathways, when glucose levels are low. Whether activation of gluconeogenesis limits the efficacy of anti-glycolytic interventions in lung cancer cells, is at present unknown. Thus, we explored the metabolic changes of lung cancer cells treated with a glycolysis inhibitor using stable isotopic tracing. Also, we explored the effects of simultaneous inhibition of glycolysis and the initial enzyme of gluconeogenesis, namely phosphoenolpyruvate carboxykinase (PEPCK, PCK2), in 2D and 3D in vitro models.

Methods
Glycolysis was blocked by 2-deoxyglucose (2-DG), a widely studied glycolytic inhibitor. Gluconeogenesis was either inhibited using the PEPCK-inhibitor Axon1165 or by targeting PCK2 via shRNA mediated silencing or CRISPR-Cas9 mediated knock-out (KO). The effect of dual inhibition was assessed by measuring the growth of lung cancer spheroids and proliferation rate of cultured lung cancer cells. To investigate the metabolic changes, control or PCK2 KO lung cancer cells were treated with 2-DG and fully 13C-labeled glucose or glutamine and stable isotopic tracing was performed using gas-chromatography mass-spectrometry (GC-MS) analysis.

Results
Inhibition of glycolysis resulted in suppression of lung cancer spheroid growth and reduction of the proliferation rate in lung cancer cells. These effects were enhanced by combined inhibition of glycolysis and gluconeogenesis. Stable isotopic tracing experiments showed that 2-DG treatment results in decreased labelling of pyruvate, lactate, alanine and TCA-cycle intermediates from 13C6-glucose. Also, the overall levels of the glycolytic intermediate phosphoenolpyruvate (PEP) were significantly lower in cells treated with the glycolytic inhibitor. However, with increasing doses of 2-DG, enhanced PEP labeling from 13C5-glutamine occurred, clearly showing that a certain proportion of PEP was derived from gluconeogenesis instead of glycolysis. In the PCK2 KO cells, this increase could not be observed. Instead, these cells showed higher labelling of PEP from 13C6-glucose compared to control cells in the presence of 2-DG. These results indicate that lung cancer cells facing glycolysis inhibition attempt to preserve PEP levels by using the initial step of gluconeogenesis mediated by PCK2. In absence of this enzyme, increased PEP formation from glycolytic precursors occurs, despite glycolysis inhibition.

Conclusion
Simultaneous inhibition of glycolysis and gluconeogenesis may represent a promising approach to circumvent the metabolic flexibility of lung cancer cells. Therefore, the underlying mechanisms behind the observed interaction need to be studied more extensively to understand the interplay of glycolysis and gluconeogenesis in lung cancer cells.

Keywords : lung cancer, metabolism, 2-DG, gluconeogenesis, glycolysis

P57 - The protective role of glutamine synthetase in β-catenin driven HCC

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Being the most common type of primary liver cancer and the fourth leading cause of cancer-related deaths worldwide, hepatocellular carcinoma (HCC) remains a huge global burden with few therapeutic options. 40% of HCCs present activating β-catenin mutations and as a result an overexpression of its downstream positive target, the glutamine synthetase (GS). Despite the long-time established use of GS as a biomarker for β-catenin-activated HCC, its exact role in this type of cancers is still a controversy and needs to be clearly identified.

Our team has previously developed a mouse model to constitutively activate the Wnt/β-catenin pathway in the liver by knocking-out the tumor suppressor gene Apc, using a Cre-loxP system, and leading to tumors mimicking human HCC. To study the role of GS in this subgroup of tumors, we performed a Cre-loxP-based double knock-out (DbKO) mouse model invalidated for both Apc and Glul, encoding for GS. According to the dose of adenovirus-Cre injected, Apc-floxed and/or Glul-floxed mice either developed tumors in few months (tumoral model, low dose) or harbored a pan-lobular activation of β-catenin signaling and subsequent overexpression of GS in all hepatocytes (pretumoral model, high dose). Tumor development was followed by ultrasonography (PIV platform, Institut Cochin). At sacrifice, activation of β-catenin pathway was confirmed by qPCR and immunohistochemistry and the proliferation rate was assessed by the Ki67 staining. The global gene expression was assessed by RNA seq and then validated by qPCR and western blot in ApcKO and DbKO tumors and pretumoral livers.

Pretumoral ApcKO livers displayed dysfunction in several metabolic pathways with accumulation of α-ketoglutarate and glutamine in particular. However, they died around 10 days after β-catenin activation due to hepatic encephalopathy associated with accumulation of ammonia in the brain. Survival of pretumoral DbKO (n=9) was dramatically decreased compared to ApcKO mice (n=16). This worsening phenotype was not related to increased hyperammonemia, but rather correlated with increased amino acid and alpha-ketoglutarate levels. Concerning the tumor model, ApcKO mice developed tumor overexpressing GS, around 9 months post-injection. A faster tumor initiation was observed in DbKO mice (n=17) compared to ApcKO mice (n=13). RNA-seq results also showed that genes that were differentially expressed between ApcKO and DbKO tumors were involved in pathways known to be regulated by glutamine, such as nucleotide synthesis, glutathione antioxidation, and immune response. Some of these genes were then validated by qPCR or western blot.

Altogether, our results support that glutamine plays a protective role in β-catenin-activated HCCs from a more deleterious phenotype since the early steps of liver carcinogenesis. We now need to decipher in more details the underlying mechanisms involved in this protective activity given the diverse roles that glutamine plays in metabolism, cell signaling and epigenetics.

Keywords : glutamine, HCC, beta catenin, mouse

P58 - Targeting MTHFD2 via the polyamine pathway in prostate cancer

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Prostate cancer (CaP) is a major public health issue, and treatments for advanced stages are not efficient enough. Cancer cells can adapt their metabolism to provide energy and cofactors for tumor growth, in response to various stresses and chemotherapies. In this context we are studying the polyamine metabolism and its interaction with the folate cycle through its regulation by MTHFD2. The folate cycle represents an interface with the synthesis of nucleotide and glutathione, NADPH production, DNA replication, RNA methylation and translation.

We observed that MTHFD2 half-life is only about a dozen of hours and that its degradation does not rely on the proteasome. Our results also indicate that different polyamines inhibitors decrease MTHFD2 protein levels between twelve and twenty four hours. Interestingly, after three hours of treatment with a polyamine inhibitor (GC7) MTHFD2 mRNA levels start to rise to anticipate the decrease of protein levels, which suggests that a sensing mechanism might take place. We also observed that knocking down DHPS, DOHH and especially EIF5A (siRNA) do not affect MTHFD2 protein levels, which indicate that its regulation relies on a different mechanism.

MTHFD2 is a key regulator of the folate pathway happening in the mitochondria. Thus, the pharmacological inhibition of the polyamine pathway would affect the mitochondrial metabolism independently of the hypusination pathway and EIF5A. Our results suggest a new regulation of MTHFD2 via the polyamine pathway. Identifying this regulation would provide a new therapeutic window against CaP.

Keywords : Tumor metabolism, polyamine pathway, one carbon cycle, mitochondria, MTHFD2

P59 - Targeting the cell and non-cell autonomous regulation of 47S synthesis by GCN2

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Introduction
Nutrient availability is a key determinant of tumor cell behavior. While nutrient-rich conditions favor proliferation and tumor growth, scarcity, and particularly glutamine starvation, promotes cell dedifferentiation and chemoresistance. Here, we link ribosome biogenesis adaptation to the nutritional environment and cell fate. Our data unveil that the stress kinase GCN2 maintains ribosome biogenesis plasticity according to the nutritional environment, ensuring cell survival and proliferation.

Material and Methods
2D and 3D models of colon adenocarcinomas (cancer cell lines and primary tumor cells) and tightly-defined experimental settings mimicking the intratumor heterogeneity of nutrients availability were used in vitro. A pharmacological inhibitor and RNA interference were used to study the impact of GCN2 inhibition. Ribosome biogenesis and nucleolar homeostasis were characterized by northern blotting and immunofluorescence staining. Cell viability assays and live-cell imaging were performed to investigate the impact on cell fate. Molecular mechanism was explored by RNA sequencing, western blotting of mTORC1 and autophagic markers (P-S6, P-4EBP1, P-Ulk1, LC3, p62) and monitoring of autophagic flux.

Results and Discussions
Under metabolic stress, we show that the amino acid sensor GCN2 represses the expression of the precursor of ribosomal RNA, 47S ribosomal RNA (rRNA). In this condition, the blockade of GCN2 triggers cell death by an irremediable nucleolar stress and subsequent TP53-mediated apoptosis in patient-derived models of colon adenocarcinoma. In nutrient-rich conditions, GCN2 activity supports cell proliferation, independently of the canonical ISR axis. Indeed, impairment of GCN2 activity prevents nuclear translocation of the methionyl tRNA synthetase (MetRS) participating in the transcription of 47S rRNA in the nucleolus. This event leads to a nucleolar stress, inhibition of mTORC1 pathway and the induction of a protective autophagic flux. Finally, inhibition of the GCN2-MetRS axis drastically improves the cytotoxicity of RNA polymerase I inhibitors, including the first-line chemotherapy oxaliplatin, on patient-derived colon adenocarcinoma tumoroids.

Conclusion
Our data thus reveal that GCN2 differentially controls the ribosome biogenesis according to the nutritional context. Furthermore, pharmacological co-inhibition of the two GCN2 branches and the RNA polymerase I activity may represent a valuable strategy for elimination of proliferative and metabolically-stressed colon adenocarcinoma cell.

P60 - Glycine decarboxylase maintains mitochondrial protein lipoylation to support tumor growth

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Cancer cells develop remarkably distinct metabolism compared to that of normal cells. Evidence for alterations in metabolic activity of malignant cells goes back almost a century ago to the discovery of the 'Warburg effect'1.
The recent resurgence of interest in the field of cancer metabolism involved numerous findings of metabolic alterations in a variety of pathways in cancer cells, oncogenes and tumor suppressors regulating metabolism, and specific oncogenic mutations in metabolic genes2,3.
A major current challenge is characterizing metabolic alterations that may be therapeutically targeted to selectively slow or halt tumor growth4,5.
A metabolic system that is highly dysregulated in cancer is one-carbon (1C) metabolism. This system encompasses the folic acid and methionine cycles and mediates the transfer of 1C units to support the biosynthesis of RNA and DNA, as well as methylation of DNA and proteins.
The importance of 1C metabolism in cancer was discovered ~70 years ago when antifolate drugs, such as methotrexate, were used in the treatment of leukemia. However, these drugs have many side effects due to the significant role of THF, the central carrier of 1C moieties, in a variety of healthy tissues. Recent studies revealed major alterations in tumor expression of 1C metabolic genes, leading to an upsurge of interest in cancer 1C metabolic research6.
While the importance of serine as a mitochondrial and cytosolic donor of folate-mediated 1C units in cancer cells has been thoroughly investigated, a potential role of glycine oxidation remains unclear.
In Shlomi's lab we developed an approach for quantifying mitochondrial glycine cleavage system (GCS) flux by combining stable and radioactive isotope tracing with computational flux decomposition. We find high GCS flux in hepatocellular carcinoma (HCC), supporting nucleotide biosynthesis. Surprisingly, other than supplying 1C units, we found that the proper function of GCS is important for maintaining protein lipoylation and mitochondrial activity.
Genetic silencing of glycine decarboxylase, GLDC, inhibits the lipoylation and activity of pyruvate dehydrogenase and impairs tumor growth.

Considering the physiological role of liver glycine cleavage, our results support the notion that tissue of origin plays an important role in tumor-specific metabolic rewiring7. Our findings suggest GLDC as a novel drug target for HCC, calling for the development of novel chemical inhibitors for GLDC and follow up pre-clinical studies.

Keywords : One-carbon (1C) metabolism, glycine cleavage system (GCS), GLDC (glycine decarboxylase) , hepatocellular carcinoma (HCC), lipoylation, PDH (pyruvate dehydrogenase).

P61 - Role of de novo synthesis of pyrimidine in breast cancer formation

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Introduction
Breast cancer is one of the most common cancers globally and the most frequent cancer in women. Detailed understanding of the biology of breast cancer can help treatment of the disease. This includes spatial arrangement cells, morphological characteristics of their nuclei, and the dynamics of proliferation.
Normal cells are engaged in regulated physiological responses to provide substrates for basic cellular processes. In contrast, cancer cells undergo a complex metabolic switch to increase energy production and biosynthetic processes to sustain cell growth and proliferation. Pyrimidines are building blocks of DNA and RNA and their fast formation is a prerequisite for cancer cell proliferation their survival and proliferation. Therefore, inhibition of de novo pyrimidines synthesis may represent a potential therapeutic strategy for breast cancer.
The main goal of this study was to determine the role of de novo pyrimidine with focus on its rate-limiting enzymes dihydroorotate dehydrogenase (DHODH) in individual stages of breast cancer tumorigenesis from normal to pre-neoplastic and malignant lesions.

Materials and methods
We used transgenic FVB/n c-neu mice that spontaneously form HER2-high breast carcinomas at 5-7 months after birth. Tumor formation was monitored twice a week by palpation and tumor size was assessed using calipers. Morphology of the tumors was identified by H&E staining. Cell division was checked using 5-ethynyl-2-deoxy-uridine (EdU). Normal tissues and tumors were evaluated for expression of DHODH using immunohistochemistry.

Results
Of 58 mice, 34 formed tumors, the incidence being 59%. The number of tumors and the age at which they appear exhibited significant variation. On average, mice in this study developed tumors at 7 months of age. Tumors that were detected in the thoracic region present 65% of all tumors, followed by the cervical region (19%).
In the early stages of tumors with the size <15 mm3, we observed the histologic structure of ductal hyperplasia and atypical hyperplasia. When the tumors reached ~35 mm3, we observed the presence of ductal carcinoma in situ. As tumors reached ~60 mm3, we detected combination of ductal hyperplasia, atypical hyperplasia, ductal carcinoma in situ, invasive ductal carcinoma, and invasive lobular carcinoma. Immunostaining revealed that DHODH expression and EdU levels were higher in the different tumor stages compared to normal mammary gland, including the earlier stage of tumor formation.

Conclusion
Our findings indicate that targeting DHODH from the early stage of carcinogenesis can be a viable druggable option to delay or prevent breast cancer initiation and progression.
This research is supported by grant GAUK 392222.

P62 - Metabolic networks regulated by mut-p53: an integrated approach to design new therapeutic strategies

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Introduction and Objectives

P53 stands out as a major tumor suppressor, highlighted by the high prevalence of somatic p53 mutations in various cancer types and the significant predisposition to multiple early-onset cancers in Li-Fraumeni Syndrome (LFS) patients carrying germline p53 mutations. Here, we generated datasets concerning the metabolic functions of two clinically relevant hot-spot Mut-p53 variants (R248W and R175H) commonly identified in human cancers. The aim of this work is to uncover mutp53-specific metabolic networks and the functions of upstream regulators in these pathways.

Methods

We profiled mouse embryonic fibroblasts (MEFs) harboring conditional knock-in (cKI) alleles (R245W or R172H) of mutp53, either individually or in combination with the Mdm2, Mdm4, or E4f1 cKO alleles. Validation of mut-p53 expression was carried out through the introduction of Cre recombinase, while disruption of bona fide transcription of p53 target genes, including Mdm2 and p21, was confirmed upon exposure to DNA damage and nutlin treatment. We employed RNA-seq to characterize the gene expression profile and NMR to analyze the exometabolome in cell culture supernatants, thereby determining secretion/uptake rates of key metabolites.

Results

Our analysis of p53-deficient MEFs revealed an unexpected role of p53 in the transsulfuration pathway and taurine metabolism. Our find-

ings indicate that p53 inactivation and the expression of the p53R245W or p53R172H mutants impair the induction of Cystathionine b-synthase (CBS) by the DNA-damaging agent doxorubicin and reduce mRNA levels of Fmo2, Fmo3, Fmo4, and Fmo6, which are associated with antioxidant functions. These data suggest a pivotal role for p53 in the intricate metabolic network encompassing serine/glycine metabolism, the transsulfuration pathway, and taurine metabolism, all contributing to maintaining a balanced redox state.

Discussion / Conclusion

Our results suggest that p53 is implicated in the proper channeling of serine into the transulfuration pathway and taurine metabolism.
We will evaluate if the role of the p53 pathway in this channeling can be used to define specific metabolic signatures and new cancer biomarkers of p53-deficient tumors. In addition, we aim to develop new Li-Fraumeni Syndrome mouse models featuring inducible loss of heterozygosity (LOH) to investigate the effects of timing LOH on tumor spectrum and survival in animals harboring mutant p53R172H or p53R245W, two of the most frequently mutated sites in LFS patients. We are willing to address important unsolved questions in the cancer field related to the metabolic functions of Mut-p53 and the role of regulators of the p53 pathway.

Keywords : p53 pathway, Mut-p53, transsulfuration pathway, taurine metabolism, Li-Fraumeni Syndrome

P63 - Ketogenic diet enhances the anti-tumor effect of mifepristone in chemical-induced breast cancer

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Introduction: Breast cancer is the most common diagnosed cancer in females accounting for 13.3% of all new cancer diagnosis in the European region in 2020. Germline BRCA1 mutation and/or prolonged or higher exposure to the sex steroid hormone progesterone are known key drivers for poor prognostic breast cancers. Moreover, evaluation of the hormone receptor status in breast cancer patients forms the basis for any personalized therapy. In this study, we used a chemical-induced, immune-competent breast cancer mouse model to elucidate a potential enhancement of the already known anti-tumor effect of mifepristone (MIF) -a progesterone receptor modulator- in combination with a low-carbohydrate, high-fat ketogenic diet (KD).

Methods: Female BALB/c mice were fed either a control (CTRL) or a KD (ratio of fat to carbohydrate + protein of 4:1). After one-week, mammary tumors were chemically induced by subcutaneous implantation of 50 mg slow-release medroxyprogesterone pellets (MPA), followed by oral administration of 1 mg 7,12-dimethylbenz[a]anthracene (DMBA) 5-6 times over 6 weeks . On the same day of MPA implantation, mice also received either a 3 mg slow-release MIF pellet or placebo. Blood parameters (glucose and beta-hydroxybutyrate [BHB]), tumor incidence, and tumor growth were monitored. Furthermore, we developed and validated a targeted high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) approach to track the distribution of MIF and metapristone (MET, an active metabolite of MIF), in plasma and several organs. Targeted metabolomics will be performed in plasma and tumor tissue using the MxP® Quant 500 kit (biocrates).

Results: MIF pellet implantation led to its metabolization into metapristone and to a systemic distribution in the organs with highest MIF levels in breast tissue followed by uterus, breast tumor tissue and liver whereas spleen showed the lowest levels.
MIF effectively delayed breast cancer tumorigenesis, resulting in a significant prolongation of both tumor-free and overall survival. The combination of mifepristone with KD further increased tumor-free and overall survival. Application of KD alone also significantly delayed tumor occurrence compared to mice fed with the control diet, although it failed to significantly slow tumor growth and subsequently to increase overall survival. As expected, KD induced ketosis in mice while blood glucose levels remained unaffected. Effects of the combination treatment on plasma and tumor metabolomes will be presented.

Conclusion: Our results show that the KD is able to enhance the anti-tumor effect of MIF. Targeted HPLC-MS/MS measurements indicate an accumulation of MIF and its active metabolite metapristone at the desired side of action, namely breast tissue. Our data underpin the importance of complementary nutritional approaches in combination with anti-cancer drugs for cancer prevention and cancer treatment.

Keywords : breast cancer, ketogenic diet, mifepristone, prevention, metabolomics

P64 - Integrative omics analysis to elucidate potential antitumor mechanisms of a ketogenic diet in melanoma

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Introduction: Dietary interventions, in particular the use of a low-carbohydrate, high-fat ketogenic diet, are highly attractive approaches to target the metabolic vulnerabilities of tumor cells. Using metabolic profiling, we have recently shown that human melanoma cells engrafted into mice presented distinct metabolomes partially independent of genetic driver mutations, such as BRAF and NRAS. Moreover, we demonstrated that treatment of these genetically and metabolically heterogeneous melanoma xenografts with ketogenic diet effectively reduced tumor growth. Targeted metabolomics of plasma and tumor samples revealed distinct alterations in amino acid and lipid metabolism induced by the ketogenic diet.

Methods: In order to study the effect of the ketogenic diet on gene expression in melanoma and to integrate different omics data sets, we analyzed the transcriptome of xenografts.

Results: RNA sequencing revealed that ketogenic diet induced changes in gene expression in melanoma, however, distinctly throughout different melanoma xenograft models, potentially indicating that most metabolic regulations induced by ketogenic diet occur on the post-translational level. Interestingly though, enrichment analysis revealed that the ketogenic diet influenced two pathways, namely ´extracellular matrix-receptor interaction´ and ´axon guidance´ consistently throughout melanoma subtypes. Moreover, key metabolic signaling pathways associated with melanoma progression, including PI3K-Akt, AMPK and MAPK signaling pathways, were regulated by the ketogenic diet in at least one of the melanoma subtypes.

Conclusion: Further correlation analysis of gene expression levels, pathways and metabolite concentrations will help us to understand the crosstalk between cancer-related transcriptional regulators, metabolism and the tumor microenvironment and, potentially, to identify possible mechanisms underlying the anti-proliferative activity of the ketogenic diet.

Keywords : melanoma, ketogenic diet, metabolomicis, RNAseqq

P65 - Metabolic rewiring in pyrimidine synthesis deficient lung cancer cells

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Abstract:
Introduction: Non-small cell lung cancer (NSCLC) is an aggressive cancer characterized by complex metabolic adaptations that boost survival in a nutrient poor environment. Dihydroorotate dehydrogenase (DHODH) is an essential enzyme of the pyrimidine de novo synthesis (DNS) pathway that provides pyrimidine nucleotides needed for proliferation. Disruption of pyrimidine DNS is detrimental to rapidly proliferating cancer cells, but current DHODH inhibitors in cancer suffer from lack of efficacy. We hypothesized that the therapeutic efficacy of pyrimidine DNS blockade might be limited by metabolic rewiring of DHODH-inhibited cancer cells and/or by upregulation of alternative salvage pathways.

Materials and methods: To map metabolic dependencies of DHODH-deficient NSCLC cells we used HKP1 murine lung adenocarcinoma, where we deleted DHODH gene by CRISPR/Cas9. We used auxotrophy measurements and a metabolism-focused loss of function pooled CRISPR screen to pinpoint pathways functionally relevant for proliferation of DHODH Knockout (KO) NSCLC cells. Seahorse XFe96 extracellular flux analyzer was used to determine the impact on energy metabolism.

Results: The results obtained so far indicate that DHODH KO in HKP1 cells compromise proliferation in vitro without affecting mitochondrial oxygen consumption or the glycolytic rate. Medium supplementation studies revealed that exogenous uridine, but no other nucleoside, rescued proliferation of DHODH KO cells in vitro. DHODH KO HKP1 cells are able to produce orthotopic tumors in syngeneic mice, albeit with a delay.

Conclusion: We found no direct link between de novo pyrimidine synthesis and energy metabolism in HKP1 cells. While uridine rescues proliferation of DHODH KOs in vitro, the factors that enable proliferation of DHODH KO tumors in vivo are a subject of ongoing investigation.

Keywords : CRISPR screen, NSCLC, DHODH, de novo pyrimidine synthesis

P66 - Deciphering the Interplay between SUMOylation and Metabolism in Acute Myeloid Leukemias

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Acute Myeloid Leukemias (AML) are severe haematological malignancies, characterized by an abnormal hematopoiesis in the bone marrow. Their treatment is largely based on intensive chemotherapy with however high relapse rate and dismal prognosis, in particular in the elderly (5-year survival below 20%). It is therefore critical to better understand the mechanisms of AML resistance to therapies and to identify new therapeutical targets. We have shown that SUMOylation, a post-translational modification related to Ubiquitylation, plays a critical role in AML response to chemotherapies (1) as well as differentiation (2) and epigenetic therapies (3). In particular, we recently demonstrated that TAK-981, a first-in-class inhibitor of SUMOylation, has a promising anti-leukemic effect both in vitro and in vivo AML models (3). Considering the tight links between metabolism and AML response to therapies and that we identified more than 200 metabolic proteins/enzymes as SUMO targets in AML, our objective is to decipher if and how SUMOylation and metabolism cooperate to regulate AML response to treatments.
We first assessed the impact of TAK-981 on the respiratory activity of AML cells using Seahorse experiments, enabling the simultaneous quantification of mitochondrial oxidative metabolism and glycolysis. Our findings revealed divergent responses to SUMOylation inhibition among different AML cell lines. Following 24 hours of TAK-981 treatment, most AML cell lines tested exhibited increased energy metabolism, characterized by elevated glycolysis and mitochondrial respiration, except for MOLM14 cells, which displayed reduced basal respiration and glycolysis. Extracellular metabolomics analysis corroborated these observations by showing decreased lactate levels in the media of TAK-981-treated MOLM14 cells but not in U937 cells. Additionally, SUMOylation inhibition by TAK-981 induced mitochondrial fission across cell lines, while overall mitochondrial mass and membrane potential remained in general unaffected, except for MOLM14 cells where mitochondrial integrity was lost after 24 hours of treatment.
These findings suggest that SUMOylation plays a role in the control of metabolism of AML cells. Our current investigations aim at elucidating the mode of action of TAK-981 and the specific characteristics of the AML cell line subgroups under study. Finally, AML cells exhibit dysregulated metabolism and SUMOylation, suggesting that a combination of SUMOylation inhibitors, such as TAK-981, and metabolic modulators could enhance AML treatment response and mitigate chemoresistance.

Keywords : Acute Myeloid Leukemia, SUMOylation, Mitochondria, Metabolism

P67 - Inhibition of choline metabolism in a murine AITL preclinical model reveals a new treatment option for AITL

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Angioimmunoblastic T cell lymphoma (AITL) is a malignancy with very poor survival outcome, in need of new more specific therapeutic

regimen. The drivers of malignancy in this disease are CD4+ follicular helper T cells (Tfh). Since the metabolic requirements of these malignant Tfh cells were not yet elucidated, we leveraged our previously established AITL mouse model by crossing metabolomic and proteomic data of murine AITL cells. Strikingly, the mAITL Tfh cells were highly dependent on the second branch of the Kennedy pathway, the choline lipid pathway, responsible for the production of the major membrane constituent phosphatidylcholine. Moreover, gene expression data from Tfh cells isolated from AITL patient tumors, confirmed the upregulation of the choline lipid pathway. Several enzymes involved in this pathway such as choline kinase, catalyzing the first step in the phosphatidylcholine pathway, respectively, are upregulated in multiple tumors other than AITL. Here we showed that treatment of our mAITL preclinical mouse model with the lipid metabolism inhibitor, etomoxir, significantly increased their survival and even reverted the exhausted CD8 T cells in the tumor into potent cytotoxic anti-tumor cells. Specific inhibition of Chokα confirmed the importance of the phosphatidylcholine production pathway in the neoplastic CD4+ T cells, since it irradicated almost all the mAITL Tfh cells from the tumors. Finally, the same inhibitor induced in human AITL lymphoma biopsies cell death of the majority of the hAITL PD-1high neoplastic cells. Our results suggest that interfering with the choline metabolism in AITL might represent a new therapeutic strategy for these patients.

Keywords : T-cell Lymphoma, Lipid metabolism, Metabolic inhibitor

P68 - Addiction to mitochondrial respiration of malignant Tfh cells reveals a new therapeutic target for AITL

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The regulation of metabolism in T cell lymphoma is poorly understood. Transcriptomic analysis of human angioimmunoblastic T cell lymphoma (AITL) cells, allowed to identify that AITL tumor cells use mainly oxidative phorphorylation (OXPHOS) metabolism to fulfill their energetic requirement. By using our pre-clinical mouse model plck-GAPDH mimicking closely human AITL features, we confirmed that PD-1high T follicular helper (Tfh) tumor cells exhibit a reduction of glycolytic flux in vivo associated with a strong enrichment of OXPHOS metabolic signatures, high mitochondrial content and ROS accumulation. Consistent with these results, disruption of OXPHOS metabolism using an inhibitor of mitochondrial electron transport chain complex such as metformin or a ROS scavenger improved survival of AITL lymphoma-bearing mice. In vitro we confirmed a selective elimination of the PD-1high Tfh human AITL cells upon ETC inhibition. In agreement, diabetic patients suffering from T cell lymphoma, treated with metformin survived longer as compared to patients receiving alternative treatment. Taking together, our finding suggests that targeting this OXPHOS pathway might be a clinically efficient approach to inhibit lymphoma progression in AITL disease.

Keywords : T-cell Lymphoma, Mitochondrial metabolism, Metformin, PD1

P69 - Links between pyruvate metabolism and protein translation in melanoma

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We recently identified the multifunctional protein E4F1, a component of the p53 pathway, as an essential regulator of skin homeostasis through the regulation of pyruvate dehydrogenase complex (PDC)1. However, E4F1 functions in skin seem to extend beyond its implication in keratinocytes and plays a major role in the melanocytic linage. Melanocytes play a protective role against UV damage by producing melanin. To evaluate E4F1 function in melanocytes, we inactivated E4f1 specifically in these cells (E4f1cKO, TyrCreER) and noticed that these mice exhibit a hair-graying phenotype and skin pigmentation defects. This phenotype was linked to a strong decrease in the expression of MITF, a key transcription factor of the melanocytic lineage and pigmentation. At the molecular level, we showed that E4f1 depletion reduces the expression of several of its target genes involved in PDC regulation but also in protein translation, including Elp3, encoding the acetyltransferase of the Elongator complex and involved in the acetylation of transfer RNAs (tRNAs) and in translation fidelity. Disruption of this molecular network leads to the induction of an unfolded protein response and decreases expression of MITF, which ultimately results in pigmentation defects2. Interestingly, MITF expression has been shown to be deregulated in melanoma, a cancer type arising from melanocytes. Recently, we showed using mouse models of melanoma that E4f1 inactivation delays tumor development. Thus, we are currently studying how disruption of pyruvate metabolism influences the epitranscriptomic profile of melanoma cells and participates in the control of translation during tumor progression but also during resistance to therapies.

Keywords : pyruvate metabolism, translation, epitranscriptomics, melanoma, resistance

P70 - The involvement of DEC1 in compensatory mechanisms following respiratory complex I ablation in high grade serous ovarian cancer models

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High grade serous Ovarian Cancer (HGSOC) is a deadly female neoplasia with at present only partially effective treatment options. Indeed, HGSOC is often metastatic since currently there are no screening tests available for early diagnosis and patients often develop chemoresistance or present with relapse, whereby it is essential to identify new targets to improve current therapies. Targeting mitochondrial respiratory complex I (CI) has been proposed as a promising anti-cancer strategy for HGSOC too. However, on the long run, cancer cells respond to CI ablation with compensatory mechanisms that allow cells to survive [1]. DEC1 (Differentiated embryonic chondrocyte gene 1) is a transcriptional factor, known for its key role in circadian rhythms, whose over-expression in OC has been described as negatively related to prognosis [2]. The combination of glucose restriction and CI inhibition through metformin has been demonstrated to reduce tumor growth through the activation of a DEC1 physical interactor, the glycogen synthase kinase 3β (GSK3β) [3], which prompted us to investigate the potential involvement of DEC1 in OC adaptation to CI ablation. Whereas in CI-competent OC cells DEC1 protein levels are reduced under glucose restriction, suggesting a dependence of DEC1 protein levels on glucose availability, such decrease is more evident in CI knock-out (KO) models, obtained by CRISPR/Cas9 system, where metabolic stress is even more significant. Furthermore, in our CI-KO models, the reduced DEC1 expression correlates with the dephosphorylation of GSK3β, suggesting a relationship between DEC1 and GSK3β. DEC1 transcriptional levels show a significant drop in CI-KO xenografts, which may be related to the slow-down in tumor growth in vivo. Interestingly, DEC1 is also known as a transcriptional repressor of Peroxisome Proliferator Activated Receptor Gamma Coactivator 1 alpha (PGC1α), the master regulator of mitochondrial biogenesis whose increase we have shown to occur as a long-term response to CI inactivation in OC [4]. Therefore, further investigations are ongoing to clarify whether DEC1 reduction could relate to the mitochondrial biogenesis trigger observed upon CI ablation or inhibition, or to the promotion of the compensatory mechanism through which PGC1α may support neo-angiogenesis.

Keywords : ovarian cancer, DEC1, BHLHE40, metabolism, mitochondrial respiratory complex I

P71 - The role of SIRT3 in breast cancer and tamoxifen resistance

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Tamoxifen (Tam) resistance represents a major clinical issue in the treatment of oestrogen receptor positive (ER+) breast cancer patients. Despite the continuous development of new therapeutic approaches, between 30% and 50% of responsive Tam-treated patients eventually acquire resistance to the therapy. With the knowledge that Tam not only affects the oestrogen-signalling pathway, but it is also able to alter mitochondrial function, our laboratory has analysed the role of mitochondria in tamoxifen resistance. We have found that sirtuin 3 (SIRT3), the major mitochondrial deacetylase, is upregulated in both models of tamoxifen resistance, the MCF7 and T47D Tam5R cells. The relation of SIRT3 with breast cancer and specifically with tamoxifen resistance is already established, but the molecular mechanisms underpinning this fact are still unknown. Therefore, we have generated MCF7 SIRT3 overexpressing (OE) and knock-out (KO) cell lines and assessed various parameters of cellular metabolism and chemoresistance. First, we carried out an unbiased proteomics and confirmed by Western blot that SIRT3 KO cells show a downregulation of proteins related to tumour development and proliferation (KYNU and PPM1E), as well as an increase in stemness markers (NANOG, SOX2), cell migration-related proteins (CENPV) and the mitochondrial antioxidant enzyme SOD2. It is well-known that SIRT3 regulates a wide range of cellular processes among them oxidative phosphorylation (OXPHOS) and detoxification of reactive oxygen species (ROS), which makes it relevant for the proper homeostasis and function of mitochondria. Additionally, cancer cells often rely on aerobic glycolysis for energy production, increasing their glucose uptake. In that context, we have noticed an upregulation in the level of cellular ROS with low glucose media in comparison with high glucose in SIRT3 KO cells. In addition, we have detected that mitochondrial respiration is impaired in the SIRT3 OE cells in high and low glucose, while the SIRT3 KO cells show diminished respiration only in low glucose media. Furthermore, we have recently discovered mutant variants of SIRT3 in the MCF7 and T47D Tam5R cells, the M7 variant and the T1 variant. In summary, our results show that SIRT3 seems to be connected to the PI3K/AKT/mTOR pathway and participate in adaptation to changes in the tumour microenvironment and nutrient availability through modulation of mitochondrial metabolism. Together, all these changes could underlie the importance of SIRT3 in the tamoxifen-resistant phenotype.

Keywords : breast cancer, tamoxifen resistance, sirtuin-3, mitochondrial metabolism

P72 - Metabolic heterogeneity in Glioblastoma, as defined by spectroscopic MRI, drives basal and post-irradiation metabolic profiles of Glioblastoma Stem-like Cells

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Glioblastoma (GB) is the most frequent and aggressive primary brain tumor. Standard therapeutic management consists in a surgical resection, combined with radio/chemotherapy. Despite this treatment, median survival doesn't exceed 15 months, due to the highly invasive nature of these tumors, linked with chemo/radioresistance capacities. This recurrence process is partly explained by the presence of a cellular subpopulation with stem-like cells features (GB Stem-like Cells, GSC) that would facilitate relapse by resistance to conventional treatments. The use of Magnetic Resonance Spectroscopic Imaging (MRSI) in the clinical field allows to highlight the metabolic heterogeneity in GB, notably through the Choline/N-AcetylAspartate Index (CNI) measurement. MRSI analysis identifies two different subtypes of tumor metabolic areas: CNI+ (CNI≥2, metabolically active) and CNI- (CNI<2, metabolically inactive). Linked to preoperative MRSI analysis in GB, our previous results demonstrated that CNI+ areas are predictive of post-radiation relapse sites. Moreover, our previous work showed in GB patients a significant GSC enrichment in CNI+ areas compared to CNI- in the FLAIR anatomic peritumoral region. Given the major involvement of both GSC and metabolic adaptations in resistance and relapse, our work focuses on the metabolic characterization of GSC from CNI+ and CNI- areas, as well as on the impact of ionizing radiations (IR) on GSC metabolism and resistance ability, in order to better understand and target the recurrence mechanisms in these metabolically active tumor areas.

Five pairs of GSC were collected from five GB patients in CNI- or CNI+ FLAIR regions and cultured in stem conditions. In the presence or absence of a subtoxic IR dose (3Gy), metabolic fluxes were studied by Seahorse XFe24. Lactate and ATP production, as well as glucose uptake were also analyzed through dedicated bioluminescence assays. Mitochondria were purified, and enzymatic activities relative to respiratory chain complexes I, II and III were measured. Finally, RNA expression levels from several GSC pairs were analyzed by RNA sequencing and RT-qPCR.

Oxygen Consumption Rate (OCR) and Extracellular Acidification (ECAR) are significantly increased specifically in GSC CNI+, leading to the hypothesis that GSC CNI+ would rely more on a mitochondrial metabolism. Lactate production is decreased in GSC CNI+, while ATP production from mitochondria is higher, compared to GSC CNI-. Regarding the respiratory chain complexes activities, only the complex II seems to have an increased activity in GSC CNI+ specifically, while the IR treatment seems to not impact on it. However, RNA sequencing highlights an increased expression of several molecular actors involved in the mitochondrial metabolism, notably CA9 (carbonic Anhydrase 9), in GSC CNI+. Of note, the IR impact on CA9 expression in CNI+ GSC seems to differ from CNI- GSC, with a specific biphasic response between 24 and 48 hours post-IR.

CNI tumor localization seems to impact GSC metabolism differently, by increasing the mitochondrial oxidative metabolism in GSC CNI+ at

the basal level and in response to IR. This upregulated mitochondrial metabolism could potentially be linked to systematic post-treatment relapses observed in patients. CA9, even if further investigations are needed, could be an interesting molecular and metabolic target for radiosensitization.

Keywords : Glioblastoma, Stem-Cell, Metabolism, Ionizing Radiation, Mitochondria

P73 - Vitamin B5 as a Bottleneck for Oncogenic Metabolism

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Introduction:
Tumours are intrinsically heterogeneous, and it is well established that this directs their evolution, hinders their classification and frustrates therapy. New methods allow the study of the genome, transcriptome and proteome of tumours at an ever increasing depth and spatial resolution. Tumour metabolism however, despite being regarded as a promising exploitable vulnerability, is lagging behind in this development and cancers are largely classified by bulk metabolic analysis. This hampers our ability to identify and target the metabolism inherent to the most malignant areas of the tumour.

Methods:
To address this shortcoming, we developed an integrated approach of correlative mass spectrometry imaging (MSI) that combines stable isotope tracing with light microscopy, electron microscopy and MSI. Unifying different technologies this multimodal pipeline allowed us to perform in situ flux analysis whilst tracking individual metabolites at a subcellular resolution and segmenting the tumour based on oncogene expression as well as the tumour microenvironment including immune cells. We applied our method to primary breast cancers, patient derived xenografts and genetically defined murine models of breast cancer.

Results:
We identify pantothenic acid (Vitamin B5) as a key metabolite associated with the oncogene Myc in breast cancer. Areas of high Myc showed increased pantothenic acid uptake, resulting in higher levels of CoA and increased Krebs cycle activity. Surprisingly, areas of high Myc did not display overt aerobic glycolysis (Warburg metabolism), but rather shunted large amounts of both glucose and glutamine into the Krebs cycle, while Myc Low areas produced more lactate. Importantly, depriving Vitamin B5 reversed many of the Myc mediated metabolic changes, and reduced tumour growth both in murine tumours as well as patient derived xenografts.

Conclusion:
Overall, our technologies allowed us to identify PA as an important prerequisite for Myc mediated oncometabolic rewiring, which can be exploited therapeutically. Furthermore, our pipeline can be expanded to the study of spatially-resolved metabolism in many different healthy and diseased organs.

P74 - Generation of a Biological Tissue Collection of pre-clinical models to rationalize biomedical research

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Since its creation in 2008, the RHEM (Réseau d'Histologie Expérimentale de Montpellier - Montpellier Experimental Histology Network) has been using custom-developed laboratory management software (Laboratory Information Management System, LIMS) to ensure the trace-

ability of all the samples of preclinical models generated by Montpellier researchers (> 100,000 paraffin blocks). By improving this software and developing an electronic querying system, the RHEM wanted to create an experimental biobank similar to human biobanks (known as the "Biological Tissue Collection" or BTC). The BTC consists of a web portal that can be used by the entire international scientific community to consult the database. Thanks to this portal, any scientist can query the BTC according to different criteria (species, genotype, mutated gene, age, sex, organ) and request histological slides or paraffin shavings from selected blocks. The histological slides can be used for a variety of histological analyses such as simple or multiplexed immunolabelling, in situ hybridization, spatial transcriptomics, etc.

The BTC portal aims to enable researchers to (1) promote ethical and responsible research, in line with the 3Rs rule, by replacing animal experiments with the use of histological slides from blocks that have already been generated and grouped together in a valuable biological collection, (2) saving time and money by avoiding repeating experiments that have already been carried out, thereby reducing the number of animals used, and (3) encouraging scientific collaboration by sharing biological material.
The BTC website (https://btc.rhem.cnrs.fr/) was opened to the scientific community at the beginning of March 2023. Initially, it will make it possible to search 25% of the tissue database, ie more than 25,000 paraffin blocks. 99% of these come from mouse models representing 365 different genotypes. This portal will be supplemented in a close future by new versions enabling the integration of animal models that have received transplants of human/mouse cells or human tissues (Patient Derived Tumor Xenograft models), as well as other query functionalities.

This public sharing tool will provide researchers with new opportunities to study biomarker expression in tissues from rodent models of human cancers that are difficult to access or expensive. We hope that this tool will help research teams to improve their general 3Rs strategy.

P75 - XCT key role in redox homeostasis and metabolism of cancer cells

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Introduction - The membrane transporter xCT (SLC7A11) in physiological condition is responsible for the cellular uptake of cystine in exchange for intracellular glutamate being crucial in redox control. Once into cells, cystine is rapidly converted to cysteine and then catalyzed to glutathione. xCT is overexpressed in several cancer types, where by increasing metabolic demand and maintaining redox homeostasis, correlates with poor prognosis and drug resistance. In addition xCT overexpression, increasing the release of glutamate, affects tumor microenvironment favoring tumor proliferation, invasion, and metastasis. Moreover, increased glutathione levels lead to a ferroptosis cell death reduction. Taking all of these into account, xCT could be used as a potential target for tumor treatment.
In this work, we investigate the metabolic rewiring in A549 lung cancer cell line and in eight pancreatic cancer (PDAC) cell lines after xCT inhibition in order to confirm xCT role as a good target in combination with other chemotherapeutic agents.

Materials/Methods – 9 cell lines were used: one lung cancer cell line (A549) and eight PDAC cell lines (AsPC-1, BxPC-3, Capan-2, CF-PAC-1, HPAF-II, PANC-1, Panc 10.05, SW1990). xCT inhibition was obtained by transient siRNA-mediated silencing or using Erastin, a small molecule known to inhibit the system XC−. Lipid peroxidation was determined quantifying malondialdehyde (MDA) using a fluorometric assay kit. ROS and autophagy were measured by FACS analyses. Mitochondrial function has been assessed with a Seahorse XF24. Metabolomics analyses, both untargeted and in the presence of isotope tracers (such as 13C-Glucose and 13C-Glutamine), were performed using a LC-QToF-MS.

Results - xCT siRNA-mediated silencing in A549 leads to significant lower levels of intracellular metabolites involved in glutathione synthesis and high levels of MDA, confirming the role of xCT in glutathione pathway and lipid peroxidation. Silencing of xCT increases glutamine uptake and mitochondrial respiration, followed by higher levels of ATP/AMP and NAD+/NADH ratios, in an attempt to maintain redox homeostasis. xCT inhibition by erastin shows a much stronger effect, observing decreased levels of: mitochondrial respiration, ATP, NAD+/NADH ratio, most of the pathways involved in anabolic process and redox homeostasis. On the other hand, we observe higher levels of ROS and autophagy. Finally, even in PDAC cell lines, characterized by high metabolic heterogeneity, erastin treatment shows GSH pathway alteration and lipid peroxidation, but different behaviors in mitochondrial respiration and redox homeostasis pathways. Moreover, analysis of cell proliferation showed significant reduction of growth under erastin treatment after 48 hours but a proliferation rescue at late time points.

Conclusions - Erastin is a good treatment to imbalance redox control in cancer cells, even on PDAC cells, but these results suggest that single drug treatment is not enough to totally inhibit pancreatic cancer cells proliferation and might contribute to develop new strategy of metabolic rewiring. New analysis on metabolomic data will be performed to better uncover metabolic vulnerability after erastin treatment, that can be exploited as targets for additional drug treatments.

P76 - HYP'ASSAY: An ELISA for the discovery of new eIF5a inhibitors

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Introduction:
Prostate cancer (PCa) is the most common cancer in men. Patients with advanced form of the disease are doomed to develop treatment resistance. We have demonstrated that the intracellular synthesis of the polyamine pathway increases PCa aggressiveness. eIF5a is a translation factor activated by hypusination, a post translational modification dependent upon one polyamine: spermidine. eIF5a hypusination is controlled by two enzymes: DHPS and DOHH.
No hypusination inhibitor is available for clinical use. Certainly, because there is no simple assay available to measure DHPS or DOHH activity.

Materials /Patients and Methods:
We have set up a sensitive and quantitative ELISA (HYP'ASSAY) that allows to measure DHPS and DOHH activities using fusion proteins (DHPS, DOHH and eIF5a) in a cell-free reaction.
A family of molecules (APs), extracted from marine sponge, were identified, isolated, and synthesized. These APs display similarities with GC7, a DHPS inhibitor. The more efficient APs were validated on PCa cell lines.

Results:
Our HYP'ASSAY has allowed us to define new hypusination inhibitors. We have tested the ability of APs derived from sea sponges to inhibit hypusination using HYP'ASSAY. The most effective APs, AE and AF, were tested on DU145. The inhibition of the hypusination by these APs was validated in intact cells. We observed that this decrease in hypusination was associated with a decrease in proliferation and in maximal mitochondrial respiration.

Conclusion:
HYP'ASSAY is a rapid, sensitive, and quantitative assay and allow to screen for new inhibitors of the hypusination of eIF5a. This new test could allow the discovery of new molecules for PCa treatment, as well as for other pathologies in which eIF5a is involved.

Keywords : Prostate Cancer, eIF5a, hypusination, inhibitors

P77 - Metabolic enzymes essential for GB cell aggressive behaviors identified from single cell transcriptome analyses.

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Introduction
Glioblastoma is a devastating brain tumor. It is characterized by a diversity of genomic anomalies, and the ability of its cancer cell populations to adapt their behavior to the constantly changing environment of a growing tumor and to therapies. Because metabolism is an obligatory step in shaping cell behavior, we looked for metabolic activities required for sustaining two GB cell properties critical for tumor aggressiveness, motility and tumorigenicity, regardless of the diverse genetic contexts encountered in patients' glioblastoma.

Material and method
To obtain a systemic view of the metabolic pathways at play in the complex setting of fully-grown tumors as observed at diagnosis, we combined analyses of publicly available single cell RNA-sequencing data obtained from glioblastoma resections with in vitro and in vivo experimental manipulations.

Results, discussion
Data reduction based on experimentally-defined molecular signatures allowed to group cells according to their potential properties, and to identify their corresponding sets of overexpressed genes encoding metabolic enzymes. Computational analyses integrating expression network modeling or trajectory modeling disclosed metabolic enzymes essential for sustaining GB cell motile and tumor-initiating properties, respectively. The soundness of the prediction of the computational modeling was experimentally verified using a collection of human

GB cells, tissue organoids and intracerebral xenografts combined with pharmacological and genetic manipulations and a variety of cell biological and biochemical assays. As a result, the cysteine metabolism enzyme 3-Mercaptopyruvate sulfurtransferase (MPST) and the most downstream component of the polyunsaturated fatty acid synthesis pathway ELOVL2, were demonstrated to be essential for GB cell motility and tumorigenicity, respectively.

Conclusion
Our result show that specific metabolic reprogramming are linked to specific cancer cell behaviors and maintained across heterogeneous genomic landscapes. They further show the relevance of analyses of single cell transcriptomes for unraveling metabolic dependencies of cell malignancy.

Keywords : scRNA-seq, metabolism, glioblastoma, cell plasticity

P78 - Deletion of Miro1 attenuates the migratory abilities and metastatic potential of cancer cells

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Introduction: Mitochondrial localization is important to meet the energetic demands that are required for cell migration. The leading edge of migrating cells needs an adequate supply of ATP, which is supported by the re-distribution of mitochondria towards the leading edge. In the context of cancer, cell migration is a critical step in the invasion process, being the initial step of metastasis. Miro1, a Rho GTPase located in the outer mitochondrial membrane, is known to play an important role in the trafficking of mitochondria. Little is known regarding the role of Miro1 in cancer cell migration, and the underlying mechanism is poorly understood. Here we hypothesized that Miro1 regulates cell invasion and formation of metastases.

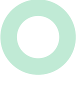
Materials and Methods: Deletion of Miro1 in B16 melanoma cells was accomplished by the CRISPR-Cas12a method. Crystal Violet was used to assess cell proliferation. Mitochondrial mass was evaluated by flow cytometry, ATP total levels by a luminescent assay, and mitochondrial respiration by Oxygraph-2k respirometer in both cell lines. Cell migration and invasion analysis were performed by evaluating focal adhesion formation (vinculin staining), the invadopodial activity (gelatin degradation assay), and by performing the spheroid formation assay. Spheroids were then used to track cell outgrowth from spheroids, as well as the rate of migration. C57BL/6J mice were injected intravenously with B16 and B16 Miro1-deficient cells, with subsequent examination of the lungs for the presence of metastatic tumors through H&E staining.

Results: B16 Miro1-deficient cells exhibited a decrease of some 20% in ATP total levels when compared to their respective controls. However, no significant differences were found in cell proliferation, mitochondrial mass, or mitochondrial respiration. Our findings also indicate that Miro-1 deficiency in B16 cells leads to a reduced invasion index (60%), track displacement (30%), and outward migration rate of cells from spheroids (45%). We also observed reduced adhesions in Miro1-deficient cells, as well as a lower ability in degrading gelatin (60% less of degraded area when compared to control). Finally, in vivo data revealed that mice injected with Miro1-deficient cells formed fewer metastatic foci in the lungs relative to parental cells.

Conclusions: Our data show that Miro1 regulates the migratory and metastatic capacity of B16 cancer cells via positioning of mitochondria and coordinating the actin cytoskeleton, which suggests that targeting Miro-1 could be a potential therapeutical approach.

Funding: Funded by the Czech Science Foundation (GA21-04607X), CZ.1.05/1.100/02.0109, RVO: 86652036, and by Portuguese national funds via Fundação para a Ciência e Tecnologia (2020.04765.BD).

Keywords : mitochondria, metastasis, Miro1



P79 - Primary cilia and prostate cancer

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Introduction:
Stabilization of Hypoxia-Inducible Factor-1α (HIF-1α) in hypoxia results in truncation of the mitochondrial voltage-dependent anion channel C-terminal (VDAC1) into VDAC1-ΔC. Recently, our team linked the regulation of ciliogenesis, processes by which the primary cilium (PC) is formed, to VDAC1-ΔC and tumor aggressiveness.
In clear cell renal cell carcinoma (ccRCC), kidney cancer, we characterized a PC signature with two markers, GLI1/IFT20. The presence of PC and the two markers Gli1+/IFT20+ was associated with more aggressive cancer and patients died faster. Our hypothesis is that such a signature can be extended to other types of cancer, including prostate cancer (PCa), which is described as no primary cilia expression. 98% of PCa patients have adenocarcinoma prostate cancer (AdPC) firstly and 20% of AdPC are induced into neuroendocrine prostate cancer (NEPC) after long-term treatment, which is the worst PCa subtype. By using TCGA cancer genomics program, we characterized, in a cohort of 550 AdPC patients, expressing the GLI1+/IFT20+ signature as well as SYP+/pRB1-, two typical markers for NEPC. Do cells from these patients express PC and are they undergoing transdifferentiation to neuroendocrine (NED) a more aggressive form of PCa?

Methods:
Normal cell line P69, prostate adenocarcinoma cell lines 22Rv1, LNCaP, DU145, PC3 and neuroendocrine cell line NCI-H660 were used as 2D cell models. 3D models include (1) RWPE1 normal cells forming acini, containing a lumen, mimicking prostate gland and (2) WPE1-NB26 tumoral cells forming a tumoroid-like structure. The presence of PC was detected by colocalization of Arl13B and acetylated α-tubulin via immunofluorescence. Gefitinib, an inhibitor of EGFR was used to restore PC as described by Khan et al. (2016). Different O2 concentrations were used to culture cells: normoxia (Nx) 21%- and hypoxia (Hx) 1%- O2.

Results:
In 2D culture, in Nx, a low percentage of PCs were detected in P69 (9%), RWPE1 (4%) and WPE-NB26 (1%) cells, none in adenocarcinoma cells but a high percentage (60%) in neuroendocrine cells. In Hx, the percentage of ciliated cells was reduced correlated with the presence of VDAC1-ΔC. Normal cells did not express PC anymore and NCI-H660 cells only expressed 10% of PC. Gefitinib increased the percentage of PC in P69 into 14% significantly, whereas RWPE and WPE1-NB26 expressed 60% of PC. Like ccRCC, cells expressing PC exhibited a Gli1+/IFT20+ signature.
In 3D, PC was detected in both acini and tumoroid formations. In the presence of gefitinib, the 3D growth of both models was inhibited, and the lumen of acini disappeared. The impact of the PC in this treatment is currently being evaluated.

Conclusion:
Our study suggests that the re-expression of PC, associated with the GLI1+/IFT20+ signature, can be used as a diagnostic basis to detect the NED early and propose a better personal therapy.

Keywords : Hypoxia, Primary Cilium, Prostate Cancer

P80 - β-blockers increase the efficacy of chemotherapy against neuroblastoma by interfering with cell metabolism, independently of their canonical targets

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Our lab has previously shown that common anti-hypertensive drugs, β-blockers, can increase the efficacy of chemotherapy in high-risk neuroblastoma, a deadly form of childhood cancer. The mechanism(s) involved remains however poorly understood. First, we showed that the enantiomers of propranolol and carvedilol (R-S) were equipotent at increasing the cytotoxic activity of vincristine, a common chemother-

apeutic agents used in neuroblastoma. Since the (R)-enantiomers have low affinity for their canonical targets, the β-adrenergic receptors, we concluded that this effect is mediated by non-canonical targets. Therefore, this project aims at revealing these non-canonical targets and better understanding the mechanism of action of β-blockers.

Using an innovative chemoproteomics approach, our first goal was to define the interactome of β-blockers in neuroblastoma. We exploited a biophysical assay called "Thermal Proteome Profiling" (TPP) coupled with quantitative mass spectrometry to evaluate the impact of β-blockers (propranolol and carvedilol) and the microtubule-targeted agent, vincristine alone or their combination, on the thermostability of the proteome of neuroblastoma cells. We applied TPP in cellulo and on cell lysates to discriminate between primary and secondary interactors. Our results highlighted well-known targets of vincristine such as tubulins, but also an enrichment in proteins involved in cell metabolism with the β-blockers and combinatorial treatments. We then performed 13C glucose and glutamine tracer experiments and showed an alteration of the glucosamine and the pyrimidine synthesis pathways as well as the tricarboxylic acid cycle under the combinatorial treatment. Additional experiments using SeaHorse® and SCENITH® technologies are currently underway to characterize the impact of the combination treatment on energy metabolism at the bulk and single-cell level. To do so we will use the neuroblastoma cells BE(2)-C and their multi resistant counterpart BE/VCR10 to map their metabolic profile and study : i) what changes in energy metabolism are associated with acquired resistance to vincristine and ii) how do β-blockers and vincristine impact energy metabolism. Finally, we will take advantage of click-chemistry, a biocompatible reaction that will allow us to couple β-blockers with an azide-coupled fluorophore to perform flow cytometry and fluorescence microscopy. We already synthetized three clickable derivatives of β-blockers and ensured that they retained their chemo-sensitizing properties. By using functional genomics and click-fluorescence approaches to perform co-localization experiments, we will now validate and characterize the identified metabolic targets of β-blockers impacting neuroblastoma biology and drug response.

Overall, our results show that β-blockers increase the efficacy of chemotherapy agents in neuroblastoma by interfering with cell metabolism, independently of beta-adrenergic receptors. This project could help better understand neuroblastoma biology and reveal novel targetable metabolic pathways, that could be further therapeutically exploited to develop new treatments and unveil biomarkers for patient selection, thus facilitating future clinical trials.

Keywords : metabolism, chemoproteomics, metabolomics, nucleotides synthesis pathway

P81 - Targeting ovarian high-grade serous carcinoma using a combination of MAT2A and PARP inhibitors

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Introduction

Ovarian high-grade serous carcinoma (HGSC) is the most aggressive subtype of ovarian cancer. PARP inhibitors have shown promising results in the treatment of HGSC by targeting tumour cells with underlying deficiencies in DNA repair, particularly in homologous recombination (HR) (1). However, the emergence of PARP inhibitor resistance poses a significant clinical challenge. MAT2A is an extrahepatic enzyme that produces the universal methyl donor, SAM, which is subsequently utilised by PRMT5 for protein methylation. MAT2A inhibition has been shown to reduce PRMT5 activity (2) and consequently reduce the accurate splicing of DNA damage repair factors such as FANCA, FANCL and ATM (3). Therefore, we hypothesised that there will be a synergistic benefit when combining Olaparib (PARP inhibitor) and AG-270 (MAT2A inhibitor) and that this effect would be greater with MTAP-loss which has been shown to be synthetically lethal with MAT2A inhibition.

Material and methods

LC-MS analysis was used to measure the effects of MAT2A inhibition on intracellular SAM levels in OVCAR4 and OVCAR5 cells. PRMT5 activity was measured via western blot (WB) for symmetric dimethylarginine (SDMA) marks on proteins. To determine whether combination treatment increased DNA damage or apoptosis we measured γH2AX (via immunofluorescence and WB) and Caspase 3/7 respectively.

Results

AG-270 reduced levels of intracellular SAM and SDMA-marked proteins, confirming inhibition of MAT2A and PRMT5. In vitro growth inhibition, levels of DNA damage and apoptosis were all greater for the combination of AG-270/Olaparib than for either therapy alone. HR and MTAP status were shown to determine the magnitude of the combination effect across a range of HGSC cell lines.

Conclusion

AG-270 and Olaparib combination can synergistically enhance therapy response in models of HGSC, including cells with intrinsic resistance to PARP inhibitors.

P82 - Exploring the kinetics of lactate metabolism in cancer cells

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Introduction
The historical notion of lactic acid as a waste product is being challenged by recent data. Lactic acidosis in the tumour microenvironment has a wide impact on cellular metabolism, directly modifying proteins and epigenetics while altering immune cell function and metastasis. However, many uncertainties remain regarding the metabolism of extracellular lactate by cancer cells, such as the possible role of substrate shuttles or channelling.

Materials and methods
13C6-glucose labelling experiments were performed in HCT116 and LS147T cells incubated with lactic acid over varying time periods. Non-stationary central carbon metabolism was modelled by fitting mass isotopomer distribution (MID) data to kinetic models within the Iso-Dyn software. To investigate the roles of the key transporters for lactic acid, monocarboxylate transporter 1 (MCT1) and 4 (MCT4), labelling experiments were repeated in LS174T cells lacking MCT1 or MCT4 in the absence and presence of the selective MCT1 inhibitor AZD3965.

Results
13C6-glucose labelling experiments showed that the presence of extracellular lactic acid decreased 13C enrichment of the TCA cycle intermediate citrate (control: 21% vs lactic acid: 6%), but not pyruvate (control: 44% vs lactic acid: 38%) in HCT116 cells. Fitting data to the canonical model for glucose carbon entry into the TCA cycle (i.e., glycolysis-derived pyruvate is transported to mitochondria before export) demonstrated a low agreement (normalized square deviation $\chi 2=73.8$). However, the introduction of two kinetically resolved pools of pyruvate and lactic acid improved the agreement ($\chi 2=24.8$). In this model, pyruvate derived from glycolysis is reduced to lactic acid before mitochondrial entry and subsequently exported across plasma membrane, while extracellular lactic acid is imported and forms a separate pool from (i.e., the uptake pool). Under these conditions, lactic acid derived pyruvate is primarily oxidised to fuel the TCA cycle. Blocking the MCT1-, but not MCT4-, dependent transmembrane transport of lactic acid in LS174T cells via knockout or AZD3965, increased 13C enrichment of pyruvate (control: 26% vs AZD3965: 51%, MCT1-/-: 50% and MCT4-/-: 22%) and citrate (control: 2% vs AZD3965: 14%, MCT1-/-: 13% and MCT4-/-: 2%).

Conclusions
Lactic acidosis induces decoupling of glycolysis from the TCA cycle, with evidence observed suggesting the presence of two MCT1-dependent, kinetically-resolved extra-mitochondrial pools of lactate/pyruvate.

Keywords : Kinetic modelling, lactate metabolism, MCT1

P83 - Expression of LPA-metabolizing enzymes affects cell migration of ovarian cancer cells.

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Lysophosphatidic acid (LPA) has emerged as a significant bioactive lipid mediator. Through interactions with specific G protein-coupled receptors on the cell surface, LPA initiates downstream signaling cascades, thus playing a crucial role in various cellular processes, including proliferation, migration and invasion. The enrichment of LPA in the tumor microenvironment has been shown to contribute to tumor progression and metastasis. Particularly in the context of ovarian cancer, LPA is reported to be overproduced, as high LPA levels are found in malignant ascites and patients' blood. In addition to the well-studied extracellular generation of LPA by autotaxin and secreted phospholipase A, there are numerous intracellular enzymes involved in LPA production. In our previous study, we demonstrated that altering the expres-

sion of the mitochondrial LPA-producing enzyme glycerol-3-phosphate acyltransferase 1 (GPAM) in different cell lines affected intracellular 16:0 and 18:1 LPA levels and cell migration. Moreover, transfecting cells with LPA led to a significant increase in intracellular LPA levels, as well as increased migration. In the present work, we aim to further investigate the role LPA metabolizing enzymes play in mediating cellular migration in ovarian cancer.
To explore the impact of intracellular LPA on cancer-related phenotypes, especially migration, we aimed to systematically investigate to what extent individual enzymes contribute to LPA metabolism and how interfering with these enzymes affects cell migration. Therefore, we used small interfering RNA molecules to transiently silence the genes of interest and analyzed their effects on migration and intracellular lipid levels. Migration was assessed using a modified Boyden chamber or scratch assay. Our targeted lipidomic panel included 16:0, 18:2, 18:1, 18:0, and 20:4 LPA, along with 81 other potentially biologically significant lipids, such as various species of phosphatidic acid (PA) and the signaling lipid diacylglycerol (DAG).
As previously reported for breast cancer cell lines, silencing GPAM in a high-grade serous ovarian cancer (HGSOC) cell line, COV318, resulted as expected in decreased migration and reduced LPA levels. Addition of external LPA to the cells did not rescue the migration phenotype. Silencing GPAM in other HGSOC cell lines, namely OAW28 and Kuramochi, consistently led to decreased cell migration, but had no impact on intracellular LPA levels. Additionally, silencing another LPA-producing enzyme, acylglycerol kinase (AGK), also led to a significant decrease in migration in all three cell lines, but LPA levels were only affected in the OAW28 cells. Constitutive overexpression of AGK in OVCAR8 and TOV112D cells increased migration but reduced intracellular LPA levels. Interestingly, silencing LPA-consuming enzymes, phospholipid phosphatase (PLPP3) and 1-acylglycerol-3-phosphate O-acyltransferase 2 (AGPAT2), in COV318 and OAW28 led, as expected, to elevated LPA levels, but surprisingly, migration was reduced.
In summary, our data strongly supports the significance of the intracellular LPA metabolic network in regulating cell functions. Notably, intracellular LPA levels alone do not account for the observed migration phenotype in all cell lines. These findings emphasize the necessity for additional research to unravel the complex interplay among key enzymes, metabolic pathways, lipid composition, and levels. Understanding these interconnected factors is essential in comprehending how they collectively govern cancer-related phenotypes.

P84 - Unraveling MELK signaling to target melanoma therapy resistance

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Melanoma is the most aggressive type of skin cancer and more than 50% of melanomas are due to BRAFV600E mutation. Frequently patients are treated with BRAF and MEK inhibitors (BRAFi+MEKi) and/or immunotherapies but achieving durable responses in only 30-50% of cases. Acquisition of resistance to treatment is a major challenge in clinic. Therefore, understanding mechanisms driving resistance is essential to identify patients most likely to respond favorably to therapy and to establish new treatments. Tumor cells alter signaling pathways to enhance nutrient utilization sustaining biosynthesis, bioenergetics, and redox homeostasis. Therefore, studying the tumor metabolic alterations is crucial for designing new therapies. The maternal embryonic leucine zipper kinase (MELK) is an oncogene overexpressed in several types of cancer, including melanoma, playing a role in tumorigenesis and malignant transformation. Some studies show that MELK is also correlated with increased drug resistance, particularly BRAFi. Aiming at targeting MELK, we developed a new inhibitor called CRO15. MELK overexpression stimulates mitochondrial metabolism, increasing OXPHOS, mitochondrial complexes activities and citrate synthase. Upon CRO15 inhibition of MELK, mitochondrial respiration and OXPHOS proteins are downregulated. MELK also leads to increased tumor growth in mice, and this effect is reversed by CRO15. We hypothesize that modulation of MELK signaling in melanoma is involved in the regulation of tumor metabolism and tumorigenicity. This supports an investigation to uncover the therapeutic potential of CRO15 as a metabolic modulator via MELK inhibition, bringing new perspectives to targeted therapies.

Keywords : : melanoma, targeted therapy, MELK, tumor metabolism

P85 - Control of fatty acid metabolism by the p53 pathway proteins.

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Introduction: p53 is a major tumor suppressor as highlighted by the high prevalence of somatic mutations in TP53 in many cancer types, and the strong predisposition to multiple early-onset cancers in LFS patients that carry germline mutations of TP53. p53 mainly functions as a transcription factor activated in response to multiple stress types that regulates the expression of genes controlling cell proliferation and senescence, DNA repair and cell death. Several laboratories also highlighted a major role of p53 in metabolism and showed that p53-associated metabolic functions contribute to its tumor suppressive activities. However, the regulators of p53-associated metabolic functions remain poorly understood. My project aims at understanding the interplay between p53 and two of its main regulators, the E4F1 and MDM2 proteins, in adaptive responses to metabolic challenges. I have a particular focus on how the p53 network is implicated in the response to nutrient deprivation, using the liver as a model organ. More specifically, I am characterizing the role of different key components of the pathway in the metabolic adaptations occurring during fasting.

Results: I generated a unique collection of genetically engineered mouse models in which p53, Mdm2 or E4f1 are specifically inactivated in hepatocytes in all possible genetic combinations (8 different models including single, double and triple knock-out (cKO)). I use these models to depict the relative implication of each protein in normal liver physiology in both fed and fasted conditions. I characterized these eight KO mouse models in parallel at the phenotypic, histological and molecular (transcriptomic, metabolomic, proteomic analyses) levels. This multi-omics strategy highlighted an important role of E4F1 in the control of the PPARα dependent response to fasting. I will present my ongoing studies aiming at understanding the molecular mechanisms by which E4F1 and p53 control the activity of p53, a process that we believe involves the regulation of p53 dimerization.

Keywords : Metabolism, Trp53, Liver, Omics, Lipids, Tissue Micro-Array

P86 - Effect of different fatty acids on ccRCC behavior and metabolism

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Introduction: Clear Cell Renal Cell Carcinoma (ccRCC) is the most common type of kidney cancer, presenting with defining characteristics of increased lipid droplets formation, loss of the VHL tumor suppressor, and low levels of fatty acid oxidation (FAO). Obesity is associated with an increase in ccRCC incidence, although obese patients respond better to different therapies, a phenomenon called as obesity pardox. The consumption of diets rich in lipids and leading to obesity has been increasing, but how the dietary composition affects ccRCC progression, or could be modulated in combination with other therapies for better responses is still unclear. We aimed to evaluate how different fatty acids (FAs) affect ccRCC behavior and metabolism.

Methods: We used 786-O and 769-P ccRCC cell lines. Palmitic acid (PA), oleic acid (OA), and linoleic acid (LA), which are saturated, monounsaturated, and polyunsaturated fatty acids, respectively, were used as stimuli for the cells at 200μM diluted in culture media with 5% FBS. Lipid droplets were evaluated by Oil Red O staining; viability was assessed by MTT; protein expression was tested by Western Blotting; Oxygen Consumption rate (OCR) and Extracellular Acidification rate (ECAR) were determined by Seahorse; survival was quantified by clonogenic assay; and mRNA was measured by RT-qPCR.

Results: All FAs increased the amount of lipid droplets compared to the control, but OA was the most potent. PA impaired the growth rate for cells compared to control, and other FAs in both cell lines, and did not increase expression of cleaved caspase-3. PA, even in a lower concentration[50μM], impaired the formation of colonies for both cell lines compared to control and other FAs. LA impaired colony formation only in the higher concentration [200μM], while OA only induced a difference for 769-P at the smaller concentration, increasing the number of colonies. OA, and mainly PA, decreased 769-P migration, at the time of 6 and 12 h. All FAs decreased OCR, mainly LA, although PA did not induce any difference in ECAR, while OA and LA also decreased ECAR.LA, and mainly PA, increased CPT1a mRNA levels in 786-O but did not induce a significant difference to 769-P, and all FAs decreased FASN in both cell lines.

Conclusion: It was demonstrated that OA was a potent inducer of lipid droplet formation, which can be protective in avoiding lipid peroxidation. On the other hand, PA and LA induced fewer lipid droplets because they are going to FAO, as we observed increased mRNA CPT1a levels, which is the rate-limiting enzyme in FAO. The decrease in OCR rate suggests that uncoupled FAO is occurring, whilst we did not observe an increase in ECAR. PA impaired potentially the formation of colonies and growth rate, and it is important to highlight that it does without inducing caspase 3 dependent programmed cell death. Taken together our data suggest that PA could be a target for ccRCC, but it is still necessary for more studies in vitro and in vivo to evaluate how an enriched lipid diet can impact a better outcome for patients.

Keywords : obesity, fatty acids, ccRCC

P87 - Metabolic rewiring in response to chemotherapy in pancreatic ductal adenocarcinoma

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As of today, surgery is the only potential cure for PDAC. However, because most patients present with either a locally advanced or metastatic disease, tumor resection can only be performed in 20% of cases. As another option, and in a palliative setting only, nearly all patients are offered chemotherapy. Treatment with FOLFIRINOX (FOX: 5-fluorouracil, oxaliplatin, irinotecan, leucovorin), leads to a higher response rate, disease-free survival, and overall survival than other treatments. However, FOX is often associated with severe toxicity and is, consequently, only considered for patients presenting with good performance status. Moreover, even among patients who are initially responders to FOX, most of them develop therapy resistance, resulting in disease progression after treatment. PDAC tumor cells (TCs) exist in an impenetrable tumor microenvironment (TME) composed of non-TCs (mainly Cancer-Associated Fibroblasts (CAFs)), extracellular matrix (ECM), that account for up to 90% of the tumor mass. CAFs secrete various components of the ECM that distorts the architecture of PDAC tissues, leading to collapsed blood vessels which impedes the efficient delivery of drugs. CAFs also exhibit diverse functions that support pancreatic tumor growth, including providing metabolic support to enable TCs proliferation in a hypoxic, and nutrient poor TME. This stromal metabolic support includes provision of amino acids to support biomass production by PDAC TCs. Metabolic reprogramming is considered a major hallmark of PDAC and is investigated for cancer diagnosis, prognosis, and treatment. However, little is known about how FOX influences PDAC TCs metabolic pathways.
A gap remains on the understanding of 1/the metabolic alterations activated in FOX-treated PDAC, 2/how FOX- altered metabolic pathways influence chemoresistance, and 3/how the TME contributes to TCs response to FOX. Thus, we aim to highlight and target the metabolic pathways and associated-key metabolic actors that are deregulated in response to FOX in PDAC and leading to either chemosensitivity or chemoresistance.

Using transcriptomic datasets of PDX mice derived PDAC samples submitted or not to FOX, we identified specific metabolic pathways of pyruvate, as its transport into the mitochondria, as involved in PDACs' FOX sensitivity. We now aim to explore on 3D cell culture systems, the metabolic pathways of interest, in connection with pyruvate, that are deregulated in PDAC in response to FOX and that are promoters of FOX sensitivity. To that end, we developed a spheroid cell culture experimental approach that integrates CAFs conditioned medium (CM) to consider the impact of the TME on pyruvate deregulated metabolic pathways upon FOX. We currently target these FOX-induced metabolic pathways using pharmacological and inducible/traceable genetic silencing tools to examine TCs' response to FOX in association with their metabolic phenotype using targeted metabolomics and metabolic tracing approaches. We intend to ultimately identify pyruvate-dependent metabolic adaptations leading to FOX sensitivity and once inhibited, the ones that are rewired and lead to FOX resistance. These metabolic candidates will be considered as prognostic markers of FOX response and will constitute therapeutic targets to improve the response of PDAC patients to FOX.

Keywords : METABOLIC REWIRING, CHEMOTHERAPY, MICROENVIRONMENT, MITOCHONDRIAL METABOLISM

P88 - Metabolic Reprogramming In Prostate Cancer: New Perspective From The Polyamines/Hypusination Pathway

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-Introduction & Objectives
Tumor development and metastatic spread require substantial metabolic plasticity. Cancer cells adapt their metabolism to meet their increased bioenergetic needs, to survive, proliferate and boost their aggressiveness. Our team is working on innovating therapeutic approaches for prostate cancer (PCa) by targeting mitochondrial metabolism. Prostate cancer cells rely on mitochondria for their bioenergetic needs. They increase the activity of Krebs cycle to produce ATP. We have come up with a new strategy to inhibit mitochondrial activity by targeting the polyamines/hypusination pathway. Hypusination is a unique post-translational modification required for the activation of the eukaryotic translation initiation factor 5A (eIF5A). This reaction is dependent on the polyamine spermidine and it is regulated by two enzymes, the deoxyhypusine synthetase (DHPS) and the deoxyhypusine hydroxylase (DOHH). Hypusination alleviate translational stalling of the ribosome at hard-to-translate motifs. It is involved in several cellular processes. However, the mechanism by which it is implicated in metabolism, tumor growth and metastasis is still unclear. Here we investigated the role of this metabolic pathway in PCa cells aggressiveness and mitochondrial metabolism.

-Materials & Methods
To explore the effect of hypusination on PCa, we worked with human PCa cell lines and patient-derived tumoroids. We inhibited hypusination by targeting the DHPS and DOHH with sh and siRNA, and pharmacologically with GC7 (inhibitor of DHPS) and ciclopirox (inhibitor of DOHH). We performed proliferation test, migration and invasion assays. To explore the role of hypusination on the metabolism of PCa cells, we performed steady state metabolomics, 13C-glucose tracing and measured real time mitochondrial respiration in 2D and 3D. We also studied mitochondrial phenotype by electron microscopy and Mitotracker. To study in depth the mechanisms regulated by hypusinated eIF5a that are involved in the aggressiveness of prostate cancer, we carried out omics experiments to study the expression of RNA, proteins and ribosomes.

-Results
Analysis of the publicaly available data bases demonstrated that the hypusination pathway is upregulated in PCa metastasis. Our results demonstrated that inhibition of hypusination decreases PCa cell growth, cell migration and invasion. Furthermore, it decreases mitochondrial respiration and disrupts the mitochondrial network. Our metabolomic and proteomic analysis revealed a reprogramming of cancer cell metabolism and an alteration of the mitochondrial respiratory chain. We identified several proteins containing ribosomal motif stalling regulated by eIF5a hypusination and we showed that these proteins control mitochondrial metabolism Interestingly, we also developed a protocol to monitor mitochondrial respiration in patient-derived tumoroids and found that the inhibition of eIF5A hypusination decreases oxygen consumption in 3D models.

-Conclusions
Metabolic plasticity is important to induce and maintain PCa aggressiveness. We show that eIF5A plays a major role in reprogramming PCa metabolism. In particular, by regulating mitochondrial metabolism, which is responsible for maintaining this aggressiveness. Our results highlight a potential therapeutic opportunity for PCa that target hypusination and could be used for clinical applications.

Keywords : Cancer, Metabolism, Mitochondria,eif5a, Organoids

P89 - Pentose phosphate pathway sustains energetic demands of leukemic B cells

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Introduction
Leukemia was one of the first tumours ever to be treated by metabolic intervention (asparaginase for B-ALL). However, leukemias may originate from distinct lineages of blood cells, for many of which metabolic vulnerabilities have not yet been mapped. Here we investigate metabolic reprogramming in the most common type of leukemia in the elderly, B-cell chronic lymphocytic leukemia (CLL). CLL cells circulate between peripheral blood and lymphoid organs where they receive proliferative cues. Continuous in situ restimulation, which can be mimicked in vitro, is associated with profound metabolic rewiring. In our work we dissect associated vulnerabilities to map metabolic pathways essential for CLL cell proliferation.

Materials and methods
We use primary CLL cells isolated from patients in a co-culture system mimicking in situ proliferative signals using culture media with different metabolite composition. Functional assays, imaging, 13C-glucose tracing, intracellular ROS measurements, RNA sequencing and CRISPR/Cas9 gene knockout by RNP electroporation have been performed to analyze metabolic requirements of proliferating CLL cells.

Results
Using cell culture media formulated with physiological concentrations of metabolites (human plasma-like media), we find that proliferating primary CLL cells have enhanced sensitivity to glucose shortage in comparison to other B cell types. We show that sustained glucose flux protects B cells against oxidative stress and sustains their optimal 3D growth properties. Sensitivity to glucose deprivation is associated with B cell inability to switch onto the use of other common physiological carbon sources. 13C-glucose tracing analysis reveals that in proliferating CLL cells glucose flux is channeled into the pentose phosphate pathway (PPP). This transition is sustained through the transcriptional regulation of PPP enzymes. Accordingly, the effects of glucose shortage on proliferation and 3D growth of CLL cells could be salvaged by restoring PPP flux. By using small molecule inhibitors and CRISPR/Cas9 knockout of metabolic enzymes we further confirm the essential role of PPP in CLL cell proliferation. Finally, evaluating CLL cases at different disease stages, we observe that dependence on PPP activity is higher in aggressive CLL, as the sensitivity to pharmacologic PPP inhibition is increased.

Conclusions
Our study highlights how the use of physiological media allows to discover metabolic dependencies in tumour cells, which can go unnoticed due to the use of culture media with supraphysiological concentrations of metabolites. Here we identify PPP as an essential node controlling CLL cell proliferation and a targetable CLL vulnerability. Further studies are needed to better understand how dependence on PPP is regulated across the clinical spectrum of CLL cases, to define metabolic target(s) for CLL treatment.

Keywords : Metabolism, leukemia, physiological media, pentose phosphate pathway

P90 - Arginine deprivation improves response to PARP inhibitors in models of ovarian cancer

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Introduction
Ovarian high-grade serous carcinoma (HGSC) is the most common and lethal subtype of ovarian cancers. Despite significant improvements in outcomes anticipated from the recent inclusion of PARP inhibitors (PARPi) as maintenance therapy in HGSC, recurrence rates remain extremely high. There is therefore an urgent need to develop novel therapeutic strategies to improve responsiveness to PARPi for HGSC patients. Arginine is a semi-essential amino acid that is involved in many physiological functions. Arginine is synthesised endogenously via the urea cycle, however, upon increased cellular demand, such as during tumorigenesis, cells increase their dependence upon extracellular sources of arginine. This creates a metabolic vulnerability that can be exploited therapeutically via an arginine depleting enzyme, ADI-PEG20, currently under investigation in several Phase I-III clinical trials. We and others have shown that arginine deprivation causes further genomic instability, which we hypothesise will increase the sensitivity of HGSC cells to PARP inhibitors.

Materials and Methods

We investigated the therapeutic combination benefit of ADI-PEG20 and the PARPi Olaparib using a series of CRISPR/Cas9 gene-edited ID8 murine models of HGSC harbouring the common genomic alterations seen in human disease. These cell lines mimic the peritoneal dissemination seen in human disease when implanted in an immunocompetent setting in vivo (female C57Bl/6 mice). Specifically we conducted two independent in vivo experiments using ID8 Trp53-/-;Pten-/- tumour bearing female C57Bl/6 mice. Immunofluorescence and western blot analysis was used to investigate the mechanistic link between arginine deprivation and genomic instability, and metabolic adaptations following arginine deprivation were measured using liquid chromatography-mass spectrometry (LC-MS).

Results

Arginine deprivation significantly reduced the growth of ID8 Trp53-/- and ID8 Trp53-/-;Pten-/- cells in vitro and increased the induction of apoptosis when used in combination with Olaparib, with a greater response observed in the ID8 Trp53-/-;Pten-/- cells. ID8 Trp53-/-;Pten-/- tumour bearing mice were then treated with either 5 IU ADI-PEG20 weekly, 50 mg/kg Olaparib daily, or a combination of both or a vehicle control, via intraperitoneal injections. Treatment of ADI-PEG20 and the combination led to a significant reduction in tumour weight and a complete reduction in the development of ascites. The combination of ADI-PEG20 + Olaparib also led to a significant increase in survival in a second in vivo study. Immunofluorescence and western blot analysis identified an increase in γH2AX foci and γH2AX expression levels, respectively, following treatment with ADI-PEG20, whilst LC-MS identified a significant reduction in nucleotide pools following treatment with arginine depleted medium in vitro.

Conclusion
ADI-PEG20 increases the genomic instability in models of HGSC and can potentiate the effects of Olaparib both in vitro and in vivo, suggesting that arginine deprivation therapy could increases the clinical responsiveness of HGSC to PARPi.

P91 - Mitochondrial Respiratory Complex II is Altered in Renal Cancer

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Introduction: Clear cell renal cell carcinoma (ccRCC) displays complex metabolic alterations driving tumor development and progression, primarily linked to the Von Hippel-Lindau (VHL) gene mutation and modulation of hypoxia-inducible factors (HIFs). Altered respiratory complex II (CII) is a negative prognostic marker in this context. Our study investigates renal cancer mitochondrial properties, with focus on CII status and assembly.

Materials/Patients and Methods: We conducted a comprehensive analysis of key mitochondrial markers in 51 tumor and adjacent normal kidney tissue from renal cancer patients, predominantly diagnosed with ccRCC characterized by expression of carbonic anhydrase-9. To explore the impact of somatic mutations in the VHL gene, we classified the patients into three groups: VHL-wild type or -mutated ccRCC and VHL-wild type non-ccRCC renal cancer. ccRCC tumor samples were also stratified based on the disease grade to evaluate grade-dependent metabolic changes. We assessed mitochondrial properties, including CII respiration, complex IV (CIV), and citrate synthase (CS) activity in fresh tissue. Protein analysis, using SDS-PAGE/western blotting (WB), identified relevant renal cancer-associated proteins and CII subunits. We also conducted native blue gel electrophoresis (NBGE/WB) on isolated mitochondria to investigate CII assembly and used an in-gel assay to assess CII activity.

Results: Our findings unveil a consistent pattern across renal tumors, demonstrating reduced CII levels and respiration compared to adjacent healthy tissue. ccRCC patients with VHL mutations exhibited elevated levels of lactate dehydrogenase A compared to non-ccRCC VHL-wild type renal cancer patients, indicative of glycolytic phenotype. Moreover, CII-dependent respiration was significantly lower in ccRCC compared to other renal cancer subtypes. Similarly, the CII status was altered, based on lower expression of the SDHA and SDHB CII subunits in ccRCC. Intriguingly, these markers along with CIV activity and mitochondrial content from tumors positively correlated with tumor grade, suggesting a link between metabolic reprogramming and tumor aggressiveness. Furthermore, CII assembly in ccRCC tumors was disrupted, resulting in the presence of CII subassembly termed CIIlow (Bezawork-Geleta et al., 2018), primarily composed of the catalytic subunit of CII, SDHA, and potentially one or two CII assembly factors, as outlined in our recent report (Sharma et al., 2023).

Conclusion: In summary, our study highlights profound variations in mitochondrial properties among renal cancer subtypes, paving the way for potential patient stratification and tailored therapeutic approaches. Additionally, the positive correlation between the CII status, the CIV activity, and tumor grade suggests their potential use as indicators of tumor aggressiveness.

Keywords : renal cancer, mitochondria, metabolism, succinate dehydrogenase, complex II assembly

P92 - Role of lactate metabolism in glioblastoma development and relapse

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Glioblastoma (GB) is the most frequent brain cancer in adult. Due to its aggressiveness and limited therapeutic options available, the median survival of GB patients reaches only 12-15 months with a high relapse rate. Indeed, GB cells exhibit a high proliferation rate, forming massive brain tumors, but also strong infiltrative capacities, allowing single cells to spread far from the tumor into the brain parenchyma.

This invasive cell population is the main responsible for the frequent GB relapse because they often escape surgical resection, the first step of GB therapy, and other treatments. As an invasive and traumatic act for the patient, resection is also a severe trouble for GB itself, disturbing both spatial and metabolic organization established between cells from the tumor core and the invasive population. Indeed, following the model of lactate shuttle described by Pellerin et al. (1) between astrocytes and neurons, we have shown that a metabolic symbiosis exists between GB tumor core and GB invasive population centered on lactate exchanges (2).

We hypothesize that tumor resection, by disturbing intratumoral metabolic organization, induces a metabolic reprogramming of the remaining GB invasive cells. By reproducing the modulations of lactate concentrations before and after resection, we show in vitro the first evidence of a metabolic switch from an oxidative pro-invasive metabolism to a glycolytic one. Mainly, we observe that the mitochondria of

invasive cells starved from their preferred substrate, lactate, are forced to downregulate their activity. This modification of mitochondrial activity is linked to increased ROS production, acting as a signaling molecule and regulating the expression of downstream metabolic actors such as hypoxic-induced factor 1α (HIF1α) and lactate metabolism pathway (LDHs, MCTs). Finally, we also observe phenotypic modulations of GB cells when starved from lactate, tending towards a re-start of proliferation, thus correlating with a tumor re-growth. The correlation between post-resection metabolic adaptation and tumor re-growth is also assessed in vivo thanks to the development of new resection models in mice implanted intracranially with GB cells.

Altogether, inhibiting the metabolic switch induced by tumor resection appears promising to enhance current treatment efficacy and prevent GB relapse.

(1) Pellerin L. et al., Dev Neurosci., 1998
(2) Guyon J. et al., EMBO Mol. Med., 2022

Keywords : Glioblastoma, Resection, Lactate, Metabolism

P93 - Targeting resistance to cancer therapy through translational control of metabolism

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Introduction
Unlike healthy cells, cancer cells harbor dysregulated signaling pathways converging to stimulate protein synthesis. Such signals remodel mRNA translation and metabolism to support neoplastic growth and cancer progression, including the development of therapy resistance (1). Protein synthesis is one of the most energy-consuming cellular processes. Recent evidence has linked the mTORC1/4E-BP/eIF4F-dependent translational regulation of metabolic genes to metabolic plasticity in oncogenic kinases-driven cancers (e.g. BRAFV600E melanoma), leading to partial resistance to metabolism-targeting therapies (e.g. combination of BRAFi and biguanides) (2). In a syngenetic murine model of AML relapse, temporary response to chemotherapy (cytarabine; araC) was accompanied by a reactivation of the mTORC1 pathway, while timed inhibition of mTORC1 improved the killing of AML (3). It is well established that chemoresistance in AML is highly dependent on mitochondrial function and OX/PHOS. All these works taken together suggest that translational programs mediated by the mTORC1/4E-BP/eIF4F axis can support resistance to therapy in various cancer models, in part by allowing cellular metabolic plasticity.

Materials and methods
Drugs: we characterized the effects of eIF4A inhibitors (CR-1-31B) in vitro and in vivo.

We performed in vitro analysis using cell lines: Pairs of BRAF-V600E mutated cells, sensitive or resistant to BRAF-inhibitors (A375, Lu1205); araC-resistant, FLT3/ITD (internal tandem duplication) - expressing AML cells (MOLM-14).

We analysed proliferation and survival; mRNA translation/protein synthesis (polysome profiling; puromycin incorporation; western blot); and did metabolic characterisation (Seahorse, GC/MS, LC/MS, nutrient deprivation).

In vivo xenograft experiments were performed using transplanted MOLM-14 cells into NSG mice.

Results
Intriguingly, we found that drugs that interfere with the translation machinery (molecules targeting the translation initiation complex eIF4F, such as inhibitors of the eIF4A subunit; eIF4Ai) are very effective in several models of resistance to therapy, in vitro and in vivo. These include BRAF inhibitor-resistant melanoma cells (both BRAFV600E or non-BRAFV600E mutated) and MOLM-14 AML cells. Classic translational targets of mTORC1/eIF4F, encoding for cell cycle and pro-survival proteins (BCL2, MCL1), are downregulated by these inhibitors. Metabolic characterization of BRAFinh-resistant melanoma cells or MOLM-14 AML cells, treated with eIF4Ai, shows a strong metabolic rewiring, particularly for TCA cycle intermediates and derivate metabolites. Bioenergetic assessment using Seahorse technology indicates a strong reduction in respiration and glycolysis. This was paralleled by a reduction in the level of proteins involved in the electron transport chain or glutamine metabolism.

Conclusion
Our studies highlight (i) the importance of the crosstalk between mRNA translation and metabolic regulation and (ii) that direct inhibition of translation represents an appealing therapeutic strategy for clinical cases of therapy resistance that are dependent on the mTORC1/4E-BP/eIF4F axis. This is particularly of interest as several translation inhibitors are being developed and currently tested in phase 1/2 clinical trials.

Keywords : metabolism, mRNA translation, eIF4A, resistance to therapy, AML

P94 - STEAP1 Interacts with STEAP2 in Ewing Sarcoma to Enhance Cellular Iron Availability and Support Metabolic Plasticity

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Background: Ewing sarcoma (EwS) is a highly aggressive bone and soft tissue tumor in children and young adults with the presence of the major disease-driving gene fusions, in 85-90% of cases - EWSR1-FLI11. EwS has one of the lowest mutation burdens among other malignant tumors. However, it is characterized by an early onset of metastasis and a poor outcome for patients with advanced stages of the disease.
Six-transmembrane epithelial antigen of the prostate 1 (STEAP1), a member of the STEAP membrane-bound protein family of transient metal reductases with high expression levels in EwS and other malignancies2. STEAP1 is a known target of EWSR1-FLI1 that increases invasiveness, tumor growth, and metastasis of EwS cells due to the oxidative stress-induced phenotype3. However, a precise functional role of STEAP1 in EwS remains elusive.

Methods and Results: We utilized formaldehyde cross-linking of STEAP1 protein complexes followed by immunoprecipitation and mass spectrometry analysis to functionally characterize STEAP1 and identified another member of the STEAP family, STEAP2. We supported the STEAP1-STEAP2 interaction using a proximity ligation assay and co-immunoprecipitation.
Genetic silencing of STEAP1 and STEAP2 led to lower intracellular iron and copper levels, implying STEAP1's metal reductase function in the presence of STEAP2. As a result, the high levels of STEAP1 and STEAP2 expression sensitize EwS cells to induction of ferroptosis upon treatment with ferroptosis inducers. Furthermore, we discovered that STEAP1 KO cells have a reduction in mitochondrial membrane potential, with a significantly reduced mitochondrial labile iron pool. The subsequent analysis of the mitochondrial activity of STEAP1 KO cells showed altered rates of oxygen consumption and glycolysis, as well as impaired assembly of supercomplexes and individual complexes of the electron transport chain. These perturbations in mitochondrial activity cause the generation of reactive oxygen species and increased rates of mitophagy upon the loss of STEAP1. Targeted metabolomics identified a depletion of numerous central carbon metabolism metabolites in STEAP1 KO cells, including the intermediates of glycolysis and the pentose phosphate pathway. Moreover, using the ex vivo pulmonary metastasis assay, we showed that the loss of STEAP1 and STEAP2 decreases the metastatic capabilities of EwS, and additional treatment with an iron chelator, deferoxamine, potentiates this effect.

Conclusion: In this study our group for the first time demonstrated in vitro interaction between two members of the STEAP protein family in the cancer cells. Moreover, this interaction elucidated a previously debatable role of STEAP1 in transition metals homeostasis, and provides alternative therapeutic strategies for EwS management through the manipulation with tumor intracellular iron levels, such as iron-chelating agents or compounds that promote ferroptosis. Finally, STEAP2, as well as STEAP1, are membrane-bound proteins with abundant expression on the cell surface of cancer cells and low levels of expression among normal tissues. Therefore, they can be utilized as promising candidates for developing targeted therapies for EwS patients, including ADCs and mono/bispecific antibodies.

Keywords : Ewing sarcoma; cancer metabolism; STEAP metalloreductases; metabolic plasticity

P96 - Dissecting metabolic and mRNA translation adaptations driving resistance to ICI: focus on the arginine synthesis pathway

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Despite the breakthrough that represent immune checkpoint inhibitors (ICI) in melanoma treatment, their efficacy is now reaching a plateau. Understanding molecular mechanisms driving resistances is essential to identify patients susceptible to respond favorably to ICI and explore strategies preventing or abrogating resistance. ICI being the first class of treatment targeting the crosstalk between immune and tumors cells, understanding the complex interactions in the tumor microenvironment (TME) is crucial to improve clinical response. Elevated resources consumption by cancer cells and limited vascularization often lead to a TME poor in nutrients and oxygen, driving competition for access to the resources between cancer and stromal cells. Thus, manipulating tumor metabolism appears as a new strategy to improve response to immunotherapies and bypass resistance. Using metabolomic and fluxomic we have identified, in melanoma resistant to ICI metabolic alterations at the corner of urea cycle and de novo pyrimidine synthesis and especially arginine synthesis upregulation. Interestingly, the limiting enzyme of arginine synthesis pathway, ASS1, is upregulated in sample from patient resistant to ICI compare to patient that respond. Thus, arginine being depleted in TME, our hypothesis is that this increase of the arginine synthesis capacities can render resistant cells less auxotroph given then and advantage to proliferate and bypass anti-tumor immunity. Based on these preliminary data, the aims of our project are to dissect these alterations to understand how metabolic reprogramming drives ICI resistance and determine if these metabolic alterations can serve as biomarkers and/or as new targets to bypass resistance.

Altogether, our project, using arginine synthesis deregulations in melanoma cells resistant to ICI as proof of concept, will allow us to identify new prognostic markers and new therapeutic targets for patient with metastatic melanoma resistant to ICI.

P96 - Does aberrant glutamate metabolism in melanoma affect dendritic cell function?

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Tumor immunity is negatively regulated by metabolites in the tumor tissue. Metabolic reprogramming impacts the activation and maturation of dendritic cells (DC). We work on the transgenic melanoma mouse model tg(Grm1)EPv, which spontaneously develops melanoma due to an overexpression of the metabotropic glutamate receptor 1 (Grm1) in melanocytes. This aberrant glutamate metabolism might besides driving tumor formation, also affect immune cell function. Therefore, we study the metabolic changes in progressing EPV melanoma and possible effects on DC and T cell responses.

Screenings of the tg(Grm1)EPv mice for metabolic changes at different stages of disease with LC-MS technology revealed a decrease in glutamate levels, ATP, TCA cycle, and glycolysis metabolites as well as amino acids within progressing tumor lesions. While the concentrations decrease, we observed that advanced tumor lesions show a percentual shift towards glutamate and glutamine. The lactate to pyruvate ratio increased significantly during tumor progression. These changes might indicate a disruption of the respiratory chain and metabolic changes in the tumor microenvironment that are advantageous for the tumor cells and unfavorable for DC.
We are currently performing detailed analyses of myeloid subsets in tumors and draining lymph nodes during tumor progression with multi-color flow cytometry. While cDC2 and macrophages decrease, neutrophils and monocytes increase in the tumor. An investigation of DC precursors in the bone marrow of tumor-bearing mice and in vitro DC differentiation assays showed no difference between tg(Grm1) EPv and C57BL/6 mice.

Further investigations will focus on alterations caused by glutamate pathway inhibition both in vitro and in vivo. The acquired knowledge can benefit the design of novel therapeutic strategies for cancer patients involving potential modification of tumor glutamate metabolism. Combination therapies with inhibitors of the glutamate pathway might improve response rates in cancer patients.

Keywords : Melanoma, Glutamate, Grm1, Dendritic cell

P97 - Macrophages flexibly use variable carbon sources and activate gluconeogenesis upon glucose starvation, while maintaining their phenotype.

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Introduction/Objectives

Macrophages are present in almost all tissues of the body, in part as highly diverse subpopulations and when required replenished from bloodstream monocytes. To maintain functionality through homeostasis and disease, macrophages may need to adapt to variable nutrient conditions. Cancer cells rapidly consume glucose and as they outgrow their supply, steep glucose gradients emerge. Consequently, tumor and immune cells, including macrophages, need to adapt to glucose starvation. It is poorly understood how a limitation of glucose affects the metabolism of macrophages in their different activation states, pro-inflammatory (M1) and anti-inflammatory (M2). The key gluconeogenesis enzyme, phosphoenolpyruvate carboxykinase 2 (PCK2), supports tumor cell survival under low glucose in some cancers, allowing synthesis of glycolytic intermediates from non-carbohydrate precursors that are shunted towards various pathways. Gluconeogenesis might support macrophages as well. Whether PCK2 is functionally expressed in macrophages to promote gluconeogenesis is unknown. We investigated macrophage metabolism, with special focus on gluconeogenesis, and adaptation to variable glucose levels, using stable isotope resolved metabolomics.

Methods

Macrophages differentiated from peripheral blood mononuclear cells (PBMCs) of healthy donors, or from THP-1 cells (+/- CRISPR/Cas9 mediated PCK2 knockout), were activated with IFN-γ/LPS (M1), or IL-4 (M2), and subjected to variable glucose treatments, either consecutive with or subsequent to polarization stimuli. We analyzed PCK2 (RT qPCR, western blot), cytokine (RT-qPCR) and surface-marker (fluorescence activated cell sorting) expression, survival (cell count), and metabolism (stable isotopic labelling, gas chromatography mass spectrometry).

Results

Low glucose conditions (0.2 mM versus high glucose, 10 mM) were readily tolerated by macrophages in all activation states and irrespective of PCK2, as shown by unchanged cell numbers. Surface marker analysis revealed a reduced expression of M1 macrophage activation marker CD80 in low glucose, while M2 polarization (CD206) was unaffected. However, after activation in high glucose, subsequent low glucose treatment does not affect either polarization marker. As well, typical M1 or M2 cytokine expression levels were not altered in low versus high glucose, in either treatment (consecutive or subsequent).
We found PCK2 expressed on mRNA and on protein level in all macrophage activation states, irrespective of glucose. Using 13C5-glutamine as tracer, we detected labelled TCA cycle metabolites, phosphoenolpyruvate (PEP) and partially glycerol-3-phosphate, primarily under low glucose, showing that gluconeogenesis is activated in macrophages. CRISPR-mediated silencing of PCK2 blocked the generation of PEP from glutamine, confirming the involvement of gluconeogenesis in the formation of glycolytic intermediates in glucose-deprived macrophages. Additionally, lactate production was decreased, while glutamine oxidation and pyruvate cycling were enhanced upon low glucose, suggesting an increase in non-glucose carbon usage for the TCA cycle.

Discussion/Conclusion

Our findings suggest impaired M1 activation upon glucose starvation, as previously described in the literature. Yet, once activated, macrophages seem to maintain their polarization, irrespective of glucose. Further, macrophages functionally adapt and activate the gluconeogenesis pathway via PCK2 in response to glucose starvation, while a reduction of glycolysis occurs. This study sheds light on macrophage metabolism in different nutritional conditions, and suggests the flexible use of different carbon sources under glucose limitation, as present in the tumor microenvironment.

Keywords : Macrophages, Immunometabolism, Metabolic adaptation, Gluconeogenesis, Tumor microenvironment,

P98 - UCP2 enhances anti-tumor T cell immune function during colorectal cancer development

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Introduction: Nowadays, colorectal cancer (CRC) stands as a major public health issue. Within this tumoral context, our laboratory is interested in the study of UCP2, an inner membrane mitochondrial carrier expressed mainly in the digestive tract and immune system. UCP2 facilitates the transport of 4-carbon metabolites from the TCA cycle to regulate pyruvate oxidation in mitochondria. In a previous study, our team demonstrated in a mouse model of colorectal cancer induced by AOM-DSS treatment, that global UCP2 invalidation led to an increased number of colorectal tumors due to a metabolic shift promoting oxidative stress. However, this investigation was carried out using a model of global invalidation. Therefore, our study aims to precisely determine whether UCP2 plays a distinct role in intestinal epithelial cells and immune cells within a colorectal cancer model.

Materials and Methods: Villin-creERT2 UCP2 lox mice (Δgut UCP2) were subjected to AOM-DSS treatment and sacrificed after 25 weeks to investigate whether the specific loss of UCP2 in intestinal epithelial cells contributes to the development of colorectal cancer. As deletion in the intestine exhibited no impact on tumor development and metabolism, we examined tumor immunity in global KO mice through flow cytometry and RT-qPCR. Then, various parameters that characterize immune cell functionality were also assessed, including 3D motility, mortality, proliferation, and activation. To better unravel immune changes in UCP2-KO tumors, CD4-cre UCP2 lox mice (Δ LT) were developed to specifically invalidate UCP2 in CD4 and CD8 lymphocytes. In parallel with immune characterization, we explored whether specific loss of UCP2 in lymphocytes could potentially contribute to the metabolic reprogramming observed in global KO tumors.

Results: Our findings within colorectal tumors revealed that the global loss of UCP2 promotes pro-tumor lymphocytes at the expense of cytotoxic cells. Moreover, the characterization of CD8 lymphocytes isolated from the spleen of global UCP2-KO mice exhibited a reduction in the number of central memory lymphocytes and a decrease in CD8 motility. These results seem to be confirmed upon specific deletion of UCP2 in lymphocytes, thereby showing a compromised immune response in the absence of UCP2. Furthermore, an imbalance in oxidative stress was also measured within the tumors. Taken together, these alterations collectively provide an explanation for the observed increase in tumor initiation.

Conclusion: In summary, these data clearly establish that the pro-tumor impact from UCP2 invalidation in the AOM-DSS model of colorectal carcinogenesis is intricately tied to a reorganization of the lymphocyte-dependent immune system. Our next goal will encompass a comprehensive characterization of the newly developed Δ LT model, where we will explore in depth T cell functionality in the context of CRC.

Keywords : UCP2, Mitochondria, Colorectal cancer, Metabolism, Immunity

P99 - Effect of N-acetyl-aspartate on microglial cells metabolism

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Introduction: N-acetyl aspartate (NAA) is among the most synthesized metabolites in the central nervous system (CNS), reaching very high concentrations even close to 10 mM. It is primarily produced by neurons and cleaved by recipient cells yielding acetate and aspartate1. Clinical evidence suggests that changes in NAA metabolism are not well-tolerated: for instance, NAA levels are significantly reduced in several neuropathologies, such as neurodegenerative diseases and glioma tumors2.

CNS homeostasis is tightly regulated by intense communication between neurons and glial cells. In particular, neurons can also release metabolites into the extracellular environment capable of modifying the functions of glial cells3.
Microglia are resident immune cells of the CNS that play a central role in inflammatory processes since they can polarize into the neurotoxic (M1) and neuroprotective (M2) phenotypes. M1 microglia are responsible for pro-inflammatory processes and utilize glycolytic metabolism for rapid ATP production; M2 microglia, instead, are involved in tissue repair and anti-inflammatory functions adopting an oxidative

metabolism4.
Data on NAA physiological role in CNS is very limited and mainly concerns oligodendrocytes where NAA-derived acetyl-CoA is used for myelin synthesis. Here we report the effects of NAA on microglia metabolism and activation.

Materials and methods: Murine BV-2 microglial cell line was used as experimental model. To evaluate the effects of NAA on microglia, we treated BV-2 with exogenous NAA at the concentration of 200 μM.
To reproduce the inflammatory phenomenon, we treated BV-2 with LPS and IFNγ at the concentration of 100 ng/ml and 20 ng/ml, respectively.
HPLC and immunofluorescence analysis were used to study lipid metabolism following NAA treatment. The effects on cell metabolism and M1 polarization were evaluated by analysis of protein and mRNA levels of the main metabolic and inflammatory markers.

Results: We demonstrated that NAA stimulates the mitochondrial oxidative metabolism sustained by an increase in lipid turnover. In particular, the acetate deriving from the catabolism of NAA can replenish the cell with cytosolic acetyl-CoA which then triggers lipid synthesis used by the cell for ATP production through oxidative phosphorylation. Under this experimental condition, BV-2 cells acquire increased migration capacity and phagocytic activity. Thereafter, we started to verify whether NAA could affect LPS/IFNγ-mediated M1 polarization. We revealed that NAA was able to mitigate the expression of pro-inflammatory markers as demonstrated by the reduction of iNOS, TNF-α and IL-6.

Conclusion: We demonstrated that NAA treatment in resting microglial cells is able to reinforce oxidative metabolism, which is typical of the homeostatic microglia phenotype, and most important it is capable to buffer the inflammatory process characterizing M1 polarization.
It would be interesting to consider these data in the field of brain tumors where microglia could play a role in the tumor microenvironment.

Keywords : NAA, Microglia, Mitochondrial metabolism, Inflammation

P100 - Can metabolic reprogramming alter T cell ability to kill lung tumour cells?

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Introduction:
Immunotherapy (IO) has revolutionised the treatment of lung cancers, with long term survival now possible in patients (1). However, predicting who will not respond and understanding drivers of resistance are vital to capitalise on the effectiveness of IO treatment. Advanced lung cancers have distinct reprogramming of glucose metabolism, driven by Krasmutant specific copy gains (2). We postulate that the changes this imposes on the metabolic tumour microenvironment (TME) will alter T cell activation and function, resulting in poor response to IO. The goal of this project is to define how metabolic reprogramming might do this, and if targeting enhanced glucose metabolism in tumour cells has potential to enhance efficacy of IO in advanced tumours.

Materials & Methods:
Using a combination of in vitro and in vivo approaches, we profile the impact of metabolic reprogramming on TME using multiomic methodologies. We define the subsequent impact on the functional T cell landscape by high-parameter flow cytometry, immunohistochemical and metabolic analyses, and evaluate the impact this has to IO response in advanced GEMM models in vivo.

Results:
Here, we confirm that advanced lung tumours with enhanced glucose metabolism display poor response to anti-PD1 treatment. Mechanistically, we can demonstrate that increased glucose metabolism in advanced tumour cells significantly alters the metabolic and cytokine profiles of the TME that can actively block T cell proliferation. Inhibition of this metabolic axis, both genetically and pharmacologically, reverts this phenotype. Furthermore, by comprehensively analysing the T cell landscape we see changes in recruitment, localisation, co-stimulation and activation of distinct T cell subtypes that together promote a more immunosuppressive, “pro-tumour” landscape. We are currently evaluating metabolic strategies to revert this TME stress, switching back on a cytotoxic response to IO therapy in advanced lung cancer models.

Conclusions:
Reprogramming of the TME by glucose-addicted lung cancer cells has a dramatic impact on T cell subsets at multiple levels – recruitment, infiltration, metabolic requirements, activation, co-stimulation, and checkpoint expression – ultimately driving a tolerant TME that in turn dampens response to IO treatment. Reverting this metabolic change in cancer cells has the potential to restore T cell function and enhance IO response, therefore our work now focuses on defining best therapeutic approaches to deliver this.

P101 - Targeting mitochondrial metabolism in the crosstalk between tumor cells and their immune environment in pancreatic cancer.

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Pancreatic cancer is one of the deadliest cancers worldwide and commonly presents as Pancreatic Ductal Adenocarcinoma (PDAC). One of the hallmarks of PDAC is the metabolic reprogramming that facilitates cancer progression and metastasis. Our group revealed that, as in other cancers, mitochondria play a central role in PDAC cells for the production of energy and substrates for anabolism, promoting aberrant proliferation and resistance to treatment. In addition, we have identified vulnerabilities in mitochondrial metabolism, offering new therapeutic avenues. PDAC is also characterized by a highly immunosuppressive tumor microenvironment and dysfunctional adaptive immunity. The recent explosion of studies on immunometabolism has highlighted the key role of mitochondria in immune cell function and fate during cancer. We therefore hypothesize that mitochondria are involved in the immunosuppressive properties of PDAC. Our objective is to analyze mitochondrial metabolism in tumor-infiltrating and peripheral immune cells in PDAC mouse models and in patients.

We used the KPC (LSL-KrasG12D+;LSL-Trp53R172H/+;Pdx1-Cre) genetically-engineered mouse model. Female mice were analyzed at 5-7 months of age and compared with age-matched KC and control mice (Cre-). In addition, we compared two housing conditions: an enriched environment allowing spontaneous exercise, and a standard environment corresponding to sedentary conditions. At sacrifice, we harvested and weighed pancreas, spleen, liver, perigonadal adipose tissue and gastrocnemius muscles. Pancreas and spleens were dissociated and analyzed by spectral flow cytometry with staining using antibodies specific of different immune cell subtypes, to examine T (CD4+, CD8+, and γδ), Natural Killer (NK) and B cells. The energy metabolism status of immune cells was analyzed by co-staining with fluorescent metabolic probes to measure mitochondrial mass, mitochondrial membrane potential (MMP), mitochondrial superoxide anion and total ROS levels. We also assessed the activation status of T cells with specific markers (CD62L, CD44, and PD-1 (Programmed Death-1)).

We observed an increase in pancreas and spleen weight in KPC mice compared to KC and controls, accompanied by decrease of adipose tissue and gastrocnemius muscles weight, in accordance with the presence of a pancreatic tumor and associated inflammation and cachexia. A correlation between the number and density of CD45+ (pan-immune cell marker) cells infiltrating the pancreas and the tumor weight was observed. Also, the pancreas from the KPC mice exhibited an increase in the number of CD4+, CD8+ and γδ T, NK, and B cells in comparison with the controls. Additionally, the percentage of PD-1 + T cells was increased in mice that harbored a large tumor. Mitochondrial mass and MMP were increased in CD4+ and CD8+ T cells in KPC pancreas compared to control, mostly in PD-1 high cells. By contrast, the levels of mitochondrial superoxide anion and total ROS were unchanged. We didn't observe any impact of voluntary exercise on immune cells in the KPC pancreas, except an attenuation of mitochondrial mass increase in PD1high T cells in large tumors, which has to be further explored.

In conclusion, our data demonstrate a metabolic dysregulation in the immune cells infiltrating the pancreatic tumor, which could affect their anti-tumoral activity.

Keywords : PDAC, Immunometabolism

P102 - Dietary restriction mitigates the age-associated decline in mouse B cell receptor repertoire diversity

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INTRODUCTION:
Ageing impairs the capacity of mammals to respond to novel antigens. Ageing individuals experience a decline in adaptive immune function, manifesting with loss of B cell receptor (BCR) repertoire diversity and increased clonal expansions. Low BCR repertoire diversity is

associated with impaired antigen recognition capacity and reduced vaccination efficacy, whereas increased clonal expansions have been linked to poor health and frailty. Dietary restriction (DR) extends life- and health span in diverse animals, and initiation of DR even at older ages can recapture some of its health benefits. However, little is known about the capacity of DR to combat the age-related decline in immune function. It is therefore important to establish whether DR, initiated either early or later in life, can ameliorate the decline in BCR repertoire diversity and the elevated clonal expansions with age.

MATERIALS AND METHODS:
To investigate how ageing and DR affect the BCR repertoire, we sequenced the variable region of the BCR heavy chain of ad libitum- and DR-fed wild-type, female C3B6F1 hybrid mice during ageing. BCR sequencing was performed on spleen and ileum of mice aged 5, 20 and 24 months and where DR was applied early (3 months) or later in adulthood (16 months), to determine if DR can have acute beneficial effects later in life.

RESULTS:
We found that, in the spleen, DR initiated in young adults maintained both the BCR repertoire diversity and lower clonal expansions during ageing. Furthermore, mice subjected to DR starting at 16 months had spleen BCR diversity and clonal expansion rates indistinguishable from those with chronic DR, suggesting an acute effect of DR. In contrast to the spleen, the BCR repertoire of the ileum showed only limited changes with age and in response to either early- or later-onset DR. However, in the ileum of old mice, DR initiated in young adults increased somatic hypermutation frequency, which is the mechanism for affinity maturation of the BCR repertoire in response to antigen exposure that diversifies the repertoire, suggesting an improved capacity for antigen binding under DR. Within-individual B cell repertoire diversity and clonal expansions in the spleen were inversely correlated with the morbidity of individual mice, suggesting a contribution of these traits to health during ageing.

CONCLUSION:
In this study, we identify remodeling of BCR features in mice under both early- and later-onset DR. We show mitigation of the age-associated increase in clonal expansions and maintenance of within-individual diversity of the BCR repertoire in the spleen and ileum of mice under DR, correlating with improved mouse health. Further, we provide evidence that the splenic BCR repertoire responds to a later start of DR. Our findings also highlight the immune responsiveness of the mice where DR was initiated at 16 months of age, indicating that, even later in life, a short-term DR treatment can have beneficial effects on the adaptive immune system in mammals.

Keywords : B cell receptor repertoire, dietary restriction, late onset, ageing, mice

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P103 - Mitochondrial metabolism, the bottleneck for T cell function in chronic lymphocytic leukemia

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Introduction:
Chronic lymphocytic leukemia (CLL) is a slowly progressing incurable B-cell malignancy. Current treatment consists of targeted small molecules that either inhibit the B cell receptor pathway or activate apoptosis. Despite clinical efficacy, resistance ultimately occurs and therefore there is need of alternative therapies. Autologous-based T-cell engaging therapies, which are effective in acute B-cell leukemia, showed low efficacy in CLL due to an acquired progressive T-cell dysfunction. T cell function is tightly linked to metabolic processes, with glycolysis being essential for the acquisition of the effector phenotype, and mitochondrial function being especially relevant for memory formation and persistence. We previously found signs of impaired metabolic plasticity in T cells from CLL patients(1). However, in-depth analysis of the metabolic signature of CLL T cells, and how it differs from healthy T cells, is lacking. We aim to better understand the basis of T-cell metabolic reprogramming in order to pinpoint routes that can be manipulated to improve T cell-based therapies in CLL.

Materials and Methods:
Peripheral blood mononuclear cells (PBMCs) of age-matched healthy donors (HD) and untreated CLL patients were analyzed by flow cytometry, extracellular flux analysis, LC-MS metabolomics and 13C fractional labelling upon stimulation with αCD3/αCD28 antibodies.

Results:
Upon stimulation, T cells from CLL patients showed abnormal proliferation profile and heterogeneous expression of T cell activation markers, confirming previous studies. Extracellular flux analysis revealed an impairment to upregulate oxidative phosphorylation (OXPHOS) in all patients analyzed, whereas glycolytic rate was variable and followed the pattern of T cell activation markers. These findings pointed towards defects in mitochondrial metabolism at the basis of T cell dysfunction in CLL. Glucose, glutamine and lipids are key sources for T cell energy production and proliferation. 13C6-glucose and glutamine fractional labelling showed that metabolic routes were maintained in CLL T cells, with glucose being mostly used in the pentose phosphate pathway and converted into lactate, and glutamine being the main fuel of

the tricarboxylic acid (TCA) cycle. However, metabolomic analyses revealed a global decrease in TCA cycle intermediates and nucleotides in CLL T cells, with unaltered glycolytic intermediates. We are currently investigating whether decreased levels/activity of the enzymes involved in these pathways explain the differences in abundance.
Since both glucose and glutamine were used to fuel specific metabolic routes, we studied the dependencies on these two substrates for T cell activation and proliferation. Interestingly, we found that HD T cells were more susceptible to glucose and glutamine deprivation than CLL T cells. Of relevance, deprivation of lipids from the media significantly impacted the proliferation of both HD and CLL T cells, indicating that CLL T cells largely depend on this third crucial fuel.

Conclusion:
T cells from CLL patients have a distinct metabolic signature upon stimulation compared to healthy T cells, which is characterized by defective mitochondrial activity and different susceptibility to fuel deprivation. Despite sustained metabolic routes, dampened production of mitochondrial intermediates and nucleotides is a bottleneck for T cell function, which should be overcome in order to improve T cell function in this disease.

P104 - MTHFD2 is a key regulator of B lymphoblast energetics

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Introduction and Objectives:
Mitochondrial one-carbon metabolism involving the catabolic processing of serine is prevalently upregulated across diverse cancers and lymphoproliferative disorders. Methylene tetrahydrofolate reductase 2 (MTHFD2) is the key rate-limiting enzyme in the pathway, which produces important metabolites such as formate and glycine for nucleotide and glutathione syntheses. Curiously, both genetic ablation and small-molecule inhibition of MTHFD2 in a B cell model of post-transplant lymphoproliferative disorder (PTLD) could not be fully rescued with formate and glycine supplementation; the role of MTHFD2 in these cells remains unclear. To address this question, we adopted a multi-omics approach to uncover new dependencies in MTHFD2-deficient cells and validated these pathway hits with molecular and cell-biological assays.

Materials and Methods:
GM12878 B lymphoblastoid cells were genetically modified by CRISPR/Cas9 editing to become MTHFD2-deficient. Control cells and MTHFD2-deficient cells were interrogated with transcriptomic, proteomic, and untargeted metabolomic approaches to identify differentially expressed genes, proteins, and metabolites in order to facilitate subsequent delineation of common targets regulated by MTHFD2. To measure cellular responses to respiratory challenges, a Seahorse instrument was used to perform a mitochondrial stress test. Drug response curves were generated by treating cells with a dilution series of small-molecule inhibitors and computationally determining the IC50. To measure cell proliferation, two methods were used: (1) seeding of identical numbers of cells and machine-assisted enumeration of cells, and (2) dye dilution assays performed with a flow cytometer. Concurrently, we also monitored cell viability with a flow cytometry-based method of vital dye exclusion. Propidium iodide staining was performed to precisely determine the cell cycle defects that arose from MTHFD2 knockout. All experiments were performed in complete RPMI-1640 media supplemented with 10% heat-inactivated dialyzed fetal bovine serum and 1% penicillin-streptomycin.

Results:
Genetic disruption of MTHFD2 in B lymphoblastoid cells led to a 50% reduction in expansion rate in vitro. MTHFD2 loss was accompanied by significant related changes to the transcriptome and proteome. MTHFD2-deficient cells increased the transcription of many genes involved in oxidative phosphorylation (OXPHOS). Alterations in OXPHOS protein levels were more graded, indicating uncoupling from transcription. Seahorse flux measurements on MTHFD2-deficient cells revealed an overall enhancement in oxidative activity with a notable increase in spare respiratory capacity. Surprisingly, untargeted metabolomics of MTHFD2-deficient cells uncovered very few alterations, although there was accumulation of intracellular serine as expected. Intriguingly, oxaloacetate and two non-proteinogenic amino acids, taurine and creatine, were significantly depleted in these cells. Subsequent supplementation of MTHFD2-deficient cells with creatine restored population expansion by reducing cell death; MTHFD2 expression status did not markedly impact cellular proliferation.

Conclusion:
MTHFD2 likely supports creatine metabolism in B lymphoblastoid cells by promoting serine catabolism to produce glycine, a necessary substrate for creatine biosynthesis. Apart from delineating the elusive function of MTHFD2, our results also point to an attractive therapeutic possibility of co-targeting one-carbon and creatine metabolism for the treatment of PTLD and similar disorders.

SPEAKERS

Reprogramming metabolic crosstalk in tumors for anti-cancer treatment

Ping Chih Ho, Lausanne, Switzerland

Cancer immunotherapies that harness tumoricidal activity of tumor-reactive T cells represent a major breakthrough of current paradigm for treating cancer patents. However, the unstable immunogenicity of tumor cells and highly immunosuppressive tumor microenvironments in solid tumors present the challenges for current immunotherapies. Deciphering the underlying mechanisms utilized by tumor cells to impede tumoricidal activity of infiltrating immune cells and to reduce their immunogenicity is direly needed. Recent studies revealed that the metabolic competition over nutrients between tumor and immune cells in the tumor microenvironment causes metabolic crisis for infiltrating immune cells, especially T cells. This process impairs metabolic fitness of tumor infiltrating T cells and results in T cell dysfunction and formation of an immunosuppressive tumor microenvironment. Therefore, the intensive metabolic communication between tumor and T cells could determine the aggressiveness and immunogenicity of tumor cells. Here, I will discuss how T cell mediated immunosurveillance shapes the metabolic activity of tumor cells via an "immunometabolic editing" process. Tumor cells could acquire the edited metabolic advantages to support their unrestricted growth and immune evasion through this undefined editing process. Given that deregulated metabolic activity is hallmark of most solid tumors that contributes to the outgrowth of tumor cells, new knowledge gained from this new dimension of immunoediting will be transformative for developing new immunotherapies and metabolism targeting strategies to successfully eradicate a broad range of malignancies.

Wednesday November 22ND 2023 - 14:25
SESSION 1 - IMMUNOMETABOLISM

Mechanisms of resistance to amino acid targeting in B-cell lymphomas

Johanna Chiche, Université Côté d'Azur, C3M, Nice, France
Reverso-Meinietti J, Mouchotte A, Rubio-Patiño C, Mhaidly R, Villa E, Bossowski JP, Proics E, Grima-Reyes M, Paquet A, Fragaki K, Marchetti S, Briere J, Ambrosetti D, Michiels JF, Molina TJ, Copie-Bergman C, Lehmann-Che J, Peyrottes I, Peyrade F, de Kerviler E, Taillan B, Garnier G, Verhoeyen E, Paquis-Flucklinger V, Shintu L, Delwail V, Delpech-Debiais C, Delarue R, Bosly A, Petrella T, Brisou G, Nadel B, Barbry P, Mounier N, Thieblemont C, Ricci JE.

Malignancies characterized by an aggressive behavior continuously rely on nutritional sources (glucose, amino acids, lipids, ketone bodies) to satisfy their energy demand. L-asparaginase (ASNase) is the only clinically approved therapy targeting a specific amino acid addiction in hematological malignancies such as acute lymphoblastic leukemias (ALLs) and NK-T-cell lymphomas (NKTCL).

Despite ASNase' treatment benefits in ALLs, NKTCLs and R-CHOP refractory Diffuse Large B-cell Lymphomas (the latter, DLBCL, having only been treated with ASNase in a context of a compassional clinical protocol with promising results (1)), resistant forms frequently appear. After a complete or partial response, disease relapse by immunization against the enzyme may occur in some cases but this only represents the tip of the iceberg and much more other mechanisms of tumor adaptation to ASNase therapy remain to be discovered. The presence of remaining tumor cells holding the capacity to survive and outgrowth in an asparagine-free environment has never been fully demonstrated to date. We will discuss our latest work carried out in a preclinical model of B-cell lymphomas, in physio-pathological conditions that enabled us to identify a flexible metabolic reprogramming in tumors cells surviving ASNase-induced nutritional stress, contributing to tumors' progression during therapy.

GAPDH Expression Predicts the Response to R-CHOP, the Tumor Metabolic Status, and the Response of DLBCL Patients to Metabolic Inhibitors. Cell Metabolism. 2019. PMID: 30827861.

Harnessing tumor metabolism to overcome immunosuppression

Dr Massimiliano Mazzone

KU Leuven, Department of Oncology, Faculty of Medicine, Belgium

Anti-cancer immunotherapy has provided patients with a promising treatment. Yet, it has also unveiled that the immunosuppressive tumor microenvironment (TME) hampers the efficiency of this therapeutic option and limits its success. The concept that metabolism is able to shape the immune response has gained general acceptance. Nonetheless, little is known on how the metabolic crosstalk between different tumor compartments contributes to the harsh TME and ultimately impairs T cell fitness within the tumor. This lecture will decipher some of the metabolic changes in the TME impeding proper anti-tumor immunity. Starting from the meta-analysis of public human datasets, corroborated by metabolomics and transcriptomics data from several mouse tumors, we ranked clinically relevant and altered metabolic pathways that correlate with resistance to immunotherapy. Using a CRISPR/Cas9 platform for their functional in vivo selection, we have identified cancer cell intrinsic metabolic mediators and, indirectly, distinguished those belonging specifically to the stroma. By means of genetic tools and small molecules, we have targeted promising metabolic pathways in cancer cells and stromal cells (particularly in tumor-associated macrophages) to harness tumor immunosuppression. Finally, we went back to patient samples to assess the relevance of these metabolic networks in humans. By analyzing the metabolic crosstalk within the TME, this lecture would like to shed some light on how metabolism contributes to the immunosuppressive TME and T cell maladaptation.

Wednesday November 22ND 2023 - 15:30
SESSION 1 - IMMUNOMETABOLISM

Dissecting the role of arginine pathway in melanoma aggressiveness and therapy resistance

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Immune checkpoint inhibitors (ICI) were a revolution in melanoma treatment. However their efficacy is now reaching a plateau. Understanding molecular mechanisms driving resistances is essential to identify patients susceptible to respond favorably and explore strategies to abrogate resistance. ICI are the first class of treatment targeting the crosstalk between immune and tumor cells. Therefore, understanding the complex interactions in the tumor microenvironment (TME) is crucial to improve responses. Elevated consumption of resources by cancer cells and limited vascularization often lead to a TME lacking nutrients, driving competition for resources between cancer and stromal cells. Therefore, manipulating tumor metabolism appears to be a strategy to improve the response to ICI. Using metabolomic analysis, we have identified, in melanoma cells resistant to ICI, metabolic alterations leading to an increase arginine synthesis along with an increase of ASS1, the rate limiting enzyme of this pathway. Interestingly we have also demonstrated that ASS1 upregulation lead to resistance to ICI. Thus, because arginine is depleted in TME, our hypothesis is that this increase in arginine synthesis capacities can render resistant cells less auxotrophic, giving them advantages to bypass antitumor immunity. Supporting this hypothesis our preliminary indicate that ASS1 modulation induce an mRNA translation reprogramming. Based on these preliminary data, the aim of our project is to dissect arginine synthesis pathway alterations to understand how metabolic and translational reprogramming participate to resistance to ICI to allowing us to identify a new prognostic marker and new therapeutic targets to combat ICI-resistant melanoma.

Importance of metabolism in mast cell regulation: from allergy to leukemia.

Fabienne Brenet, PhD

Cancer Research Center of Marseille (CRCM)

Cellular metabolism controls the functions of immune cells. However, mast cell metabolic reprogramming is underexplored. Mast cells are major effectors of allergy and asthma but also key player in clonal mast cell disorders also known as systemic mastocytosis (SM) characterized by mast cell expansion in organs and activation. The consequences of these KIT-addicted neoplasms range from benign chronic manifestations to very aggressive leukemic tumors, making this receptor and its signaling an original model for discovering the metabolic shifts leading to mast cell activation but also to tumor progression.

In this study, we conducted a comprehensive investigation using mouse preclinical model, patient plasmas and bone marrow aspirates, to decipher the metabolic underpinnings underlying SM aggressiveness. Through an integrated approach combining ex vivo and in vivo analysis, we identified the metabolic vulnerabilities of advanced mastocytosis and paved the way towards the development of new, metabolism-based treatment options in this pathology difficult to treat.

Bioenergetic of Hypoxic Glycolysis controls tumors, pathogens, immunity and tissue repair - Genetic deconstruction and therapeutic perspectives

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First, we will discuss how fermentative glycolysis, a primitive hypoxic imprinted metabolic pathway present at the emergence of life is instrumental for the rapid growth of cancers, regenerating tissues, immune cells but also bacteria and viruses during infections. The ‘Warburg effect’, activated via HIF-1 and Myc, respectively in response to hypoxia and growth factors, is a Master metabolic and energetic pathway which satisfies nutritional and energetic demands required for rapid genome replication.

Second, we will present the key role of lactic acid, the end-product of fermentative glycolysis able to move across cell membranes in both directions via monocarboxylate transporting proteins (i.e. MCT1/4) contributing to cell-pH homeostasis but also to the complex immune response via acidosis of the tumour microenvironment. Importantly lactate is recycled in multiple organs as a major metabolic precursor of gluconeogenesis and energy source protecting cells and animals from harsh nutritional or oxygen restrictions.

Third, we will revisit the Warburg effect via CRISPR-Cas9 disruption of glucose-6-phosphate isomerase (GPI-KO) or lactate dehydrogenases (LDHA/B-DKO) in two aggressive tumours (melanoma B16-F10, human Colorectal adenocarcinoma LS174T). Full suppression of lactic acid production reduces but does not suppress tumour growth due to reactivation of OXPHOS. In contrast, disruption of the lactic acid transporters MCT1/4 suppressed glycolysis, mTORC1, and tumour growth as a result of intracellular acidosis.

Finally, we will briefly discuss the current clinical developments of an MCT1 specific drug AZ3965, and the recent progress for a specific in vivo MCT4 inhibitor, two drugs of very high potential for future clinical applications against cancers, bacterial and viral pathogens.

Unexpected Guardians: 5,7-Unsaturated Sterols as Cytoprotective Metabolites

Pedro Friedmann Angeli, Würzburg, Germany

Ferroptosis, a distinctive form of cell demise, has garnered significant attention not only for its potential to target specific tumor types but also for the perspectives it offers into the metabolic adaptations that tumors employ to counteract lipid oxidation. In this presentation, I will discuss recent findings unraveling a pro-ferroptotic role played by 7-dehydrocholesterol reductase (DHCR7) and an unexpected pro-survival function of its substrate, 7-dehydrocholesterol (7-DHC). Although prior investigations had hinted at the cytotoxicity of elevated 7-DHC levels in developing neurons due to their promotion of lipid peroxidation, our research illuminates a contrasting facet—namely, the ability of 7-DHC accumulation to confer robust pro-survival attributes in cancer cells. Intriguingly, 7-DHC, owing to its significantly heightened reactivity towards peroxyl radicals, emerges as an effective shield, safeguarding (phospho)lipids from autoxidation and the ensuing fragmentation. These findings were substantiated through experiments conducted on neuroblastoma and Burkitt lymphoma xenografts, wherein the accumulation of 7-DHC is shown to induce a shift toward a ferroptosis-resistant state within these tumors, ultimately culminating in a more aggressive tumor phenotype. In conclusion, our study provides compelling evidence of an unexpected anti-ferroptotic activity attributed to 7-DHC, revealing it as a cell-intrinsic mechanism that cancer cells may exploit to evade ferroptosis.

Thursday November 23RD 2023 - 09:30
SESSION 2 - DRUG RESISTANCE / FERROPTOSIS / METABOLISM

An Atlas of Ferroptosis-induced Secretomes

Silvia von Karstedt, Cologne, Germany

Cells undergoing regulated necrosis systemically communicate via the release of chemo- cytokines as well as endogenous danger-associated molecular patterns (DAMPs). Ferroptosis is a recently described iron-dependent type of regulated necrosis driven by massive lipid peroxidation. While membrane rupture occurs during ferroptosis, a comprehensive appraisal of ferroptotic secretomes and their potential biological activity has been lacking. Here, we apply a multi-omics approach to provide an atlas of ferroptosis-induced secretomes. While proteins with assigned DAMP and innate immune system function such as MIF and heat shock proteins (HSPs) were released from ferroptotic cells, this happened in the absence of bona fide inflammatory chemo- cytokines. Non-protein secretomes with assigned inflammatory function contained oxilipins including prostaglandin E2 (PGE2) as well as Methionine-cycle metabolites. Functionally, incubation of bone marrow-derived macrophages (BMDMs) with ferroptotic supernatants induced inflammatory transcriptional reprogramming resulting in enhanced cytokine secretion upon inflammatory challenge. These results define the spectrum of ferroptosis-induced secretomes and identify a biological activity with important implications for the fine-tuning of inflammatory responses.

Host and tumor metabolism driving drug resistance in acute myeloid leukemia

Jean-Emmanuel Sarry, Centre de Recherches en Cancérologie de Toulouse (Oncopole), France

Relapses caused by drug resistance is the major barrier for effective treatment of most solid tumors and hematological malignancies. In acute myeloid leukemia (AML), we showed that mitochondrial adaptation is a critical determinant of resistance to cytarabine (AraC). We proved that this is associated with an increased availability of respiratory substrates and cofactors, mitochondrial biogenesis and transfer from stromal cells, iron-sulfur cluster biogenesis, BCL2 dependency, and ROS detoxification upon inflammatory and stress responses. We next demonstrated that the ectonucleotidase CD39 and extracellular ATP promote AraC resistance by enhancing mitochondrial biogenesis and OxPHOS activity through the activation of a cAMP-/ATF4-mediated mitochondrial stress response (MSR). Interestingly, OxPHOS metabolism and MSR are also determinants of the response to IDH- and BCL2-selective inhibitors. Additionally, our group and others have identified several other markers of relapse-initiating cells (RICs) that are metabolite sensors or transporters such as CD36, SLC7A5 and SLC1A3, which underlined metabolic dialogues between resistant blasts and their microenvironment. This provided access to a broad range of respiratory substrates including fatty acids, glutamine, aspartate, lactate and/or glucose to support the energetic metabolism of RICs. More specifically, CD36 was positively associated with extramedullary dissemination of leukemic blasts in vivo and in patients. Furthermore, CD36 inhibition reduced metastasis of blasts and prolonged survival of chemotherapy-treated mice. Our ongoing work is highlighting that the role of metabolic and transcriptional trajectories of three distinct RIC populations, diverse tissue metabolomic ecosystems and the impact of ketogenic diet during the course of disease progression.

Thursday November 23RD 2023 - 11:05
SESSION 2 - DRUG RESISTANCE / FERROPTOSIS / METABOLISM

Metabolic stress in the tumor microenvironment

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PReTT group (Precinical and experimental research in thoracic tumors). IDIBELL (Institut d'Investigació Biomédica de Bellvitge), L'Hospitalet (Barcelona), Spain

Tumor cells require nutrients and oxygen at quantities that exceed their supply. This promotes secretion of pro-angiogenic molecules in order to restore balance. Additionally, nutrient avidity of tumor cells leads to an immunosuppressed environment thought to be due to nutrient competition between tumor and immune cells. We subjected a variety of tumor cell lines to nutrient restriction and studied their secretome in order to explore tissue responses driven by starvation. Cytokine arrays unveiled that starved tumor cells secrete molecules that modulate immune functions and angiogenesis. Acute starvation triggered the secretion of the neutrophil chemoatractant IL-8 and the inflammatory mediator IL-6, both of which participate in tumor growth and correlate with poor prognosis of Non Small Cell Lung Carcinoma. I will present these and other results that indicate that the hypoglycemic conditions of the nutrient microenvironment promote paracrine responses that can lead to persistent inflammation, angiogenesis and cancer immunosuppression, as well as cancer-host rewiring.

Deciphering the metabolic determinants of GBM cell adaptation to stress

Erika COSSET

Team : GLIMMER Of lIght « GLIoblastoma MetabolisM, HetERogeneity, and OrganoIds”

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Glioblastoma (GBM), a lethal brain tumor, represents one of the most common and aggressive primary brain tumors. By identifying distinct gene expression and methylation profiles, GBM have been initially stratified by various gene signatures into four molecular subtypes (Classical, Mesenchymal, Proneural, and Neural, which was later removed from this classification) with specific driver mutations, prognoses, and responses to therapy. Despite major research efforts and progress in neuroimaging, neurosurgery, chemotherapy (with temozolomide, TMZ), and radiotherapy, the profound degree of heterogeneity within and among GBM tumors represents one critical factor contributing to poor patient outcome (16 months overall survival). Recently, our results, in line with others, have highlighted the strong metabolic heterogeneity, not only between but also within GBM patients. These data pave the way for exploring and understanding; 1/the dynamic interplay between different GBM cell belonging to different subtypes 2/ the alternative cellular processes used by GBM cells to fuel themselves, 3/ the basic bioenergy metabolism profiles selected by GBM cells to survive in this harsh tumor microenvironment.

The multiple faces of two-carbon metabolism in cancer progression and therapeutics

Eyal Gottlieb

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The high metabolic demands of rapidly proliferating cancer cells require substantial quantities of metabolic building blocks. However, many tumors are situated in a metabolically challenging environment where the blood supply, and thus of oxygen and other nutrients, is low. This environment of scarcity induces global changes in gene expression patterns as cells adapt to their surroundings to meet metabolic demands. The importance of adaptive metabolism programs to tumor maintenance and progression cannot be overstated, and modulation of essential energy processes is a validated therapeutic strategy that can have robust anti-tumor effects.

The sole carbon source and precursor for fatty acid and cholesterol biosynthesis in mammalian cells is acetyl-CoA. The cytosolic pool of acetyl-CoA is mainly supplied by two different ATP-dependent reactions: (i) cleavage of citrate into oxaloacetate and acetyl-CoA by ATP citrate lyase (ACLY), or (ii) ligation of acetate and CoA by short-chain acyl-CoA synthetases (ACSSs). There are two ACSS genes: a mitochondrial isoform, ACSS1, and a cytosolic/nuclear isoform ACSS2. We demonstrated dependency on acetate and ACSS2 for tumor growth, especially under hypoxic conditions where glucose-derived pyruvate is reduced to lactate rather than oxidized in the mitochondria to acetyl-CoA by the pyruvate dehydrogenase complex (PDH). Thus, under hypoxic conditions, acetate becomes an essential carbon source, and it is captured in cells by ACSS2 to support lipid biosynthesis in the cytosol and histone acetylation in the nucleus. Based on these observations, we developed potent and specific ACSS2 inhibitors (ACSS2i), and an investigational new drug (IND). Using this compound, we further demonstrated the essential role of acetate metabolism in maintaining DNA stability, and an effective cooperation between ACSS2i and DNA damaging chemotherapeutic drugs has been demonstrated.

Glutamine addiction in the CLL microenvironment; towards therapeutic applications and a PET tracer as a novel diagnostic tool

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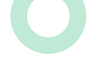
Introduction
Chronic lymphocytic leukemia (CLL) circulates between peripheral blood and lymph node (LN), where cells proliferate, and acquire drug resistance. In the clinic, emerging resistance to the Bcl-2 inhibitor Venetoclax (VEN) is a growing problem. Recently, we mapped the metabolic reprogramming in CLL LN and found that glutamine is the main fuel of TCA cycle. The main glutamine importer in proliferating human and mouse cells is ASCT2, encoded by the gene SLC1A5. Blocking glutamine import in CLL cells by the compound V9302 strongly attenuated CD40-induced resistance to VEN (Chen et al, Blood 2022). In the current study, we have investigated the role of ASCT2 and the consequences of its inhibition in human and mouse CLL cells, and healthy T lymphocytes, and explored the potential 18F-labelelled glutamine as Positron Emission Tomography (PET) tracer.

Methods
The metabolic and drug sensitivity properties of CLL cells were investigated by flow cytometry, gene expression analysis, extracellular flux analysis and metabolomics. CRISPR/Cas9 gene editing and adoptive-cell transfer experiments were performed using the mouse leukemic cell line TCL1. To enable in vivo assessment in diagnostic setting, (2S,2R)-4-fluoroglutamine (4-18F]FGln)2 was synthesized as PET tracer.

Results
Using BCR- and CD40-stimulated human CLL cells as a proxy for the LN setting, we dissected the main routes of glutamine usage. We found that in addition to its role in the TCA cycle, glutamine import is linked to mTOR signaling. The ASCT2 inhibitor V9302 impaired mTOR signaling and decreased translation of new proteins, including the anti-apoptotic proteins Bcl-XL and Mcl-1, thus explaining the reduction in VEN resistance. Of relevance for therapeutic approaches, the effector and proliferative capacity of human T cells was preserved upon V9302 treatment. This suggests that blocking ASCT2 will not affect adaptive immune responses.
The SLC1A5 gene was deleted by CRISPR/Cas9 technique in murine TCL1 cells. Adoptive cell transfer experiments of SLC1A5-KO and WT TCL1 cells injected at 50-50% ratio in recipient mice showed selective outgrowth of SLC1A5-WT cells, with no detection of the SLC1A5-KO in the spleen of overt leukemic mice. This indicates that TCL1 leukemic cells rely on SLC1A5/ASCT2 for growth.
Finally, we explored the high expression of ASCT2 and glutamine addiction in CLL from the diagnostic perspective. With the aim of improving the currently suboptimal [18F]-Fluorodeoxyglucose PET for imaging of CLL LN sites, we successfully synthesized 4-[18F]FGln. We observed rapid uptake of the 4-[18F]FGln tracer in CD40-stimulated CLL cells, which decreased in the presence of V9302, confirming specificity. Experiments to perform PET with 4-[18F]FGln in TCL1 mice to demonstrate an in vivo proof of concept are ongoing.

Conclusions
These data support a crucial role for ASCT2 and glutamine in leukemia development and VEN resistance in CLL. This provides a basis for targeting the transporter for therapeutic options and/or taking advantage of its function for diagnostic approaches using 4-[18F]FGln as PET tracer.

Keywords : CLL, microenvironment, venetoclax, glutamine, PET-tracer



Non-canonical micropeptides in rewiring mitochondria function of head and neck cancer

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Radiotherapy is the primary therapeutic option for head and neck squamous cell carcinoma (HNSCC). However, most patients develop acquired radioresistance, leading to local disease recurrence. Increasing evidence suggests that long non-coding RNAs (lncRNAs) can produce biologically active micropeptides. We comprehensively mapped lncRNA-encoded micropeptides in head and neck cancer by combining ribosome profiling and artificial intelligence-powered micropeptide prediction. By combining the CRIPSR-based tagging approach and targeted mass spectrometry-based proteomics, we validated the expression of the predicted micropeptides in a high-throughput manner. By merging the multi-omics analysis of radioresistant HNSCC cells, and clinical data from radiation-treated HNSCC patients, we identified micropeptides associated with HNSCC radioresistance. A significant portion of the identified micropeptides show mitochondrial localization and contributes to radioresistance by rewiring mitochondria function. Our results not only reveal the contribution of lncRNA-encoded micropeptides to radiation resistance in head and neck cancer but also highlight their therapeutic potential.

Thursday November 23RD 2023 - 16:55
SESSION 3 - THERAPY, TARGET AND METABOLISM

Unexpected Activation of the Bile Acid pathway! Cholesterol a key metabolite in ccRCC tumorigenesis

Clear cell renal cell carcinoma (ccRCC) incidence has risen steadily over the last decade. We and others have demonstrated that elevated lipid uptake and storage is required for ccRCC cell viability; however, the role of stored cholesterol, the most abundant component in ccRCC intracellular lipid droplets, remains unclear. We recently demonstrated that ccRCC cells acquire exogenous cholesterol through the HDL receptor SCARB1, inhibition or suppression of which induces apoptosis. Here, we show that elevated expression of 3 betahydroxy
steroid dehydrogenase type 7 (HSD3B7), which metabolizes cholesterol-derived oxysterols in the bile acid biosynthetic pathway, is essential for ccRCC cell survival. Repressing HSD3B7 expression genetically, or treating ccRCC cells with a newly identified HSD3B7 inhibitor, resulted in toxic oxysterol accumulation, impaired proliferation, and increased apoptosis in vitro and in vivo. These data demonstrate that bile acid synthesis regulates cholesterol homeostasis in ccRCC and identifies HSD3B7 as a plausible therapeutic target.

Metabolic rewiring Driving Metastasis Formation

Sarah-Maria Fendt, Leuven, Belgium

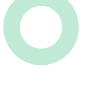
Metastasis formation is the leading cause of death in cancer patients. We find that metabolic rewiring is a liability of metastasizing cancer cells. For example, we discovered that extracellular remodeling of the metastatic niche, a process essential to metastasis formation, requires a transcriptional-independent regulation via the metabolites. Moreover, we provide knowledge on intratumor heterogeneity of metabolism and its role in pre-metastatic niche and metastasis formation. Thus, we study the metabolism of metastasizing cancer cells with the goal to define novel therapeutic strategies.

Friday November 24TH 2023 - 10:00
SESSION 4 - MICROENVIRONMENT METASTASIS

The BMP signaling differently affects stem cell functions depending on their resident tissue: from in breast early transformation to bone marrow metastatic cells dormancy

CNRS UMR5286, Inserm U1052, Université de Lyon 1, Centre Leon Bérard, Centre de Recherche en Cancérologie de Lyon, France, Department of Cancer Initiation and Tumor Cell Identity, Veronique.maguer-satta@lyon.unicancer.fr

Understanding mechanisms of cancer development is mandatory for disease prevention and management. In healthy tissue, the microenvironment or niche governs stem cell fate by regulating the availability of soluble molecules, cellcell contacts, cell-matrix interactions, and physical constraints. Gaining insight into the biology of the stem cell microenvironment is of utmost importance, since it plays a role at all stages of tumorigenesis, from (stem) cell transformation to tumor escape and metastasis. In this context, BMPs (Bone Morphogenetic Proteins), are key mediators of stem cell regulation directly or indirectly by regulating the dynamic dialogue with their residing niches. Among them, BMP2 and BMP4 emerged as master regulators of normal and tumorigenic processes. We have uncovered dysregulations of the BMP pathway in stem cells and their niche (including exposure to pollutants such as bisphenols) able to initiate breast cancer. We have modeled a chronic exposure of normal stem cells to abnormal BMP signals that lead to a cancer stem cells phenotype and disease progression. Also, we uncover a fundamental cooperation between BMP4 and TGFb2 in controlling breast cancer metastasis dormancy.



Migration, mechanosensing and metabolism in pancreatic ductal adenocarcinoma invasion and metastasis

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive types of cancer with high metastatic rates and poor prognosis. KRAS mutations are major drivers of PDAC and active KRAS triggers activation of the downstream Rho GTPase protein Rac1. Rac1 is an orchestrator of protrusion formation, migration and metastasis through its regulation of the Scar/WAVE signalling pathway. This impacts not only migration, but also macropinocytosis, a major nutrient-gathering and receptor trafficking mechanism for PDAC cells. Previously we have identified CYRI-B as a novel negative regulator of the RAC1- Scar/WAVE pathway through its interaction with active Rac1. Here, we find that CYRI-B is highly expressed in PDAC and can drive PDAC progression and an abnormal tumour microenvironment in mice. CYRI-B depleted tumours are less metastatic and the cells from these tumours are impaired in chemotaxis. Rac1 dependent localisation of CYRI-B at macropinocytic cups influences internalisation of the lysophosphatidic acid receptor LPAR1, impairing chemotaxis. Our results demonstrate a novel role of CYRI-B in PDAC onset and progression, as well as metastasis and suggest new avenues for treatments.

Friday November 24TH 2023 - 11:20
SESSION 4 - MICROENVIRONMENT METASTASIS

How glioblastoma cells adapt their metabolism when colonizing the brain ?

Antonio C. Pagano Zottola, Claire Larrieu, Joris Guyon, Mathieu Larroquette, Johanna Galvis, Audrey Burban, Andreas Bikfalvi, Macha Nikolski, and Thomas Daubon.

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Glioblastoma (GB) is a highly infiltrative brain tumor, associated with a low median survival, despite aggressive therapeutic management. Metabolic exchanges are central during tumor development. Lactate symbiosis has been described between glycolytic astrocytes and oxidative neurons, the latter consuming astrocytic lactate to fuel TCA cycle. We recently showed that a lactate symbiosis exists between glycolytic and oxidative GB cells to sustain growth and invasion (Guyon et al, 2022 ; EMBO Mol Med). We are now interrogating how cells adapt their metabolism when escaping on myelinated neurons. We show that co-cultured GB cells with neurons have higher invasive potential by rearranging their molecular and metabolic status (e.g. LDHA upregulation). Moreover, we demonstrate that GB cells destroy oligodendrocytes. We studied the role of myelin during white matter tract invasion, and GB cells exposed to myelin fragments change their metabolic status. We show that myelin stimulated GB cell invasion by activating glycolysis and mevalonate pathways.
In parallel, we developed in vivo models to reproduce clinical protocols for GB patients. By performing surgery resection, we show an impact on metabolic status when cells are not exposed to high lactate concentrations after debulking the tumor.
These studies highlight that metabolite exchanges are crucial for GB development and represent original therapeutic targets.

Metabolic Alterations in Tumor and Host in Cancer

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Ludwig Princeton Branch, Ludwig Institute for Cancer Research, Princeton University

Cancer is a metabolic disease. Oncogenic events alter tumor cell metabolism to produce building blocks and mitigate redox stress while suppressing the high energy consuming functions of normal professional cells. Tumor cells also engage nutrient scavenging pathways (e.g. micropinocytosis for extracellular nutrients and autophagy for recycling of intracellular nutrients) to sustain metabolism. In advanced cancer, factors produced by tumors drive systemic inflammation and the wasting of host tissues, particularly the dedicated nutrient stores of muscle and fat, in a process known as cachexia. Cancer cachexia is responsible for most cancer deaths, but the underlying mechanisms are unclear. Determining who cancer metabolism is altered and how tumors alter the metabolism and function of host tissues can identify new targets for cancer therapy.

Acknowledgements