Meeting report of the 4th biennial Metabolism and Cancer symposium

Nadine Abdel Hadi[1]*, Emeline Boet[2,3]*, Airelle Lahalle[4,5]*, Laura Lauture[2,3]*, Alice Refeyton[6]*, Gabriela Reyes-Castellanos[1]*, Nathalie Caplet[7], Alice Carrier[1], Laurent Le Cam[4,5], Nathalie M. Mazure[8], Jean-Ehrland Ricci[8,9], Stéphane Rocchi[8], Jean-Emmanuel Sarry[2,3], Sophie Vasseur[1], Marija Vlaski-Lafarge[6], Rodrigue Rossignol[10#], Frédéric Bost[8#]

1- Centre de Recherche en Cancérologie de Marseille (CRCM), Unité 1068, Institut National de la Santé et de la Recherche Médicale, Institut Paoli-Calmettes (IPC), Unité Mixte de Recherche (UMR 7258), Centre National de la Recherche Scientifique (CNRS), Université Aix-Marseille, F-13009 Marseille, France

2- Centre de Recherches en Cancérologie de Toulouse, Université de Toulouse, Institut National de la Santé et de la Recherché Médicale, Centre National de la Recherche Scientifique, Toulouse, France.

3- Equipe Labellisée Ligue Nationale Contre le Cancer 2018, Toulouse, France.

4- Université de Montpellier, Institut de Recherche en Cancérologie de Montpellier, Institut Régional du Cancer de Montpellier, INSERM, U1194, F-34298 Montpellier, France.

5- Equipe Labellisée Ligue Contre le Cancer, F-75013 Paris, France

6- Département de Recherche, Etablissement Français du Sang Nouvelle Aquitaine, Unité 1035 Inserm, Bordeaux, France.

7- BRIO (Bordeaux Recherche Intégrée en Oncologie) F-33076 Bordeaux, France.

8- Université Côte d'Azur (UCA), INSERM U1065, C3M, F-06204 Nice, France

9- Equipe labellisée Ligue Contre le Cancer, Nice, France

10- CELLOMET, INSERM U1211, Bordeaux University, Bordeaux, France.

*Contributed equally to the work

co-senior authors

Corresponding author: bost@unice.fr

Running title: 4th biennial Metabolism and Cancer symposium

Abbreviations: ROS, reactive oxygen species; IR, ischemia-reperfusion; TCA, tricarboxylic acid; RET, reverse electron transport; PDAC, Pancreatic Ductal AdenoCarcinoma; NAC, N-AcetylCysteine; PDCLs, patient derived cell lines; ETC, electron transport chain; TNBC, triple negative breast cancer; OXPHOS, oxidative phosphorylation; mRNA, messenger RNA; FAO, Fatty Acid Oxidation; HIF, hypoxia-inducible factor; PHDs, prolyl-hydroxylases; ADO, cysteamine (2-aminoethanethiol) dioxygenase; PARP, Poly (ADP-ribose) polymerase; *BRCA1*, *BRCA2,* breast cancer–associated genes 1 and 2; HR, homologous recombination; alt-NHEJ, alternative nonhomologous end joining repair; TPZ, tirapazamine; LSC, leukemic stem Cells; CML, chronic myeloid leukemia; HSC, hematopoietic stem cell; TKi, tyrosine kinase inhibitors; MRD, minimal residual disease; SDH, succinate dehydrogrenase; PPGL, pheochromocytoma and paraganglioma; 2-OG, 2-oxoglutarate; TET, ten-eleven translocation; 5hmC, 5-hydroxymethylcytosine; EMT, epithelial-to-mesenchymal transition; methylglyoxal, MG; SAM, S-Adenosyl-Methionine; AcCoA, AcetylCoenzymeA; METTL5, METTL6, Methyltransferase Like 5 and 6; ES cells, embryonic stem cells; m3C, 3-Methylcytidine; OGT, O-GlcNAc transferase; OGA, O-GlcNAcase; TAM, tumor-associated macrophage; HBP, hexosamine biosynthetic pathway; ETC, electron transport chain; ChREBP, Carbohydrate Responsive Element Binding Protein; HCC, Hepatocarcinoma; KEC, kidney epithelial cells; TFAM, Mitochondrial transcription factor A; PGC-1α, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPARD, PHGDH, Proliferator-activated Receptor Delta; phosphoglycerate dehydrogenase.

Keywords: Metabolism, Cancer, Mitochondria, Hypoxia, Redox, Signaling, Epigenetics

The authors declare no conflict of interest.

ABSTRACT

The 4th International meeting Metabolism and Cancer initially programed to take place in Bordeaux (France) was held virtually on May 27-29, 2021. The three-day event was followed by around 600 participants daily from 47 countries around the world. The meeting hosted 21 speakers including selected talks and a keynote lecture from the Nobel prize winner Sir Peter J. Ratcliffe (Oxford, United Kingdom). Presentations and discussions were divided in four scientific sessions: (1) Redox and energy metabolism; (2) Redox and hypoxia; (3) Metabolic profiling and epigenetic control; and (4) Signaling, fueling and metabolism in cancer and a general public session on cancer and nutrition. This report summarizes the presentations and outcomes of the 4th annual Metabolism and Cancer symposium. We provide here a summary of the scientific highlights of this exciting meeting.

Introduction

Initially programed in Bordeaux, the biennial meeting was finally virtual because of the COVID-19 pandemic. Whereas the first two days were dedicated to scientific presentations, the third day was devoted to a general public on the theme Cancer and nutrition. organized in close collaboration with the French network on nutrition and cancer (NACRe). During the two last decades multiple metabolic targets have been identified in cancer cells, it is now time to go forward with the clinical applications. The speakers envisioned the future therapeutic applications of targeting cancer cell metabolism. Here, we provide a summary of their presentations during which many new findings in the field of metabolism and cancer were highlighted, with a strong focus on redox and mitochondrial metabolism and hypoxia. A replay of the main sessions is available upon free registration @ https://www.metabolism-cancer.com/

SESSION I: Redox and energy metabolism

Mitochondrial redox metabolism: friend or foe?

In this session, the speakers illustrated how changes in mitochondrial redox metabolism are implicated in human pathologies, including cardiovascular diseases and different types of cancer, and how these alterations represent potential new targets for the development of new therapies.

“Mitochondria are the heart of the cell”, said Mike Murphy (University of Cambridge, UK), during the presentation of his work which aims at deciphering the impact of mitochondrial oxidative damage in human pathologies. Dr. Murphy discussed the role of mitochondrial reactive oxygen species (ROS) in the pathology of ischemia-reperfusion (IR) injury, which occurs mainly during stroke and heart attack. Even if reperfusion of ischemic tissues is necessary to maintain cell viability, it comes with a price to pay in terms of oxidative damage. To further address this clinically important question, Murphy and collaborators developed a

comparative *in vivo* and *ex vivo* metabolomics approach in different tissues (i.e. heart, liver, brain, and kidney). This approach demonstrated that succinate is the only tricarboxylic acid (TCA) cycle metabolite accumulating during ischemia and more importantly, it is responsible for mitochondrial ROS production during reperfusion [1, 2]. Further investigations revealed that the accumulated succinate during ischemia is highly re-oxidized by succinate dehydrogenase, leading to extensive ROS production from reverse electron transport (RET) at mitochondrial complex I [1, 2]. Murphy then raised the following question: "why does succinate accumulation/oxidation happen"? The answer is that it is not fortuitous. Indeed, pro-inflammatory macrophages reprogram their mitochondria to sustain a high membrane potential, leading to a decrease in the levels of ATP production and an increase in the generation of ROS/succinate, a process that promotes glycolysis to maintain ATP production [3]. To challenge their hypothesis *in vivo*, Murphy and collaborators used mice harboring a ND6-P25L mutation that leads to functional mitochondria, but which are impaired for ROS production by RET. They observed that ND6-P25L mice are protected against cardiac IR injury, fully demonstrating the pathological role of mitochondrial ROS production by RET through complex I during reperfusion [4]. These data suggest that succinate is a marker of ischemia and a good proxy to assess mitochondrial status. More importantly, Murphy and colleagues show that the inhibition of succinate accumulation by malonate is an efficient strategy to protect cardiomyocytes against IR injury.

When it comes to anti-cancer therapeutic strategies, non-apoptotic mechanisms of cell death such as ferroptosis represent promising strategies. Ferroptosis is an iron-dependent non-apoptotic cell death mechanism characterized by the accumulation of lipid peroxides and loss of membrane integrity. Dr Milica Vucetic (CSM, Monaco) highlighted the importance of cysteine-starvation as a potent inducer of ferroptosis in Pancreatic Ductal AdenoCarcinoma (PDAC) cells. More specifically, Dr Vucetic proposed the targeting of the cysteine transporter

xCT. Indeed, genetic invalidation of xCT in MIA PaCa-2 and Capan-2 cells sensitizes them to ferroptotic cell death, which was fully prevented via N-AcetylCysteine (NAC) or lipophilic antioxidants (vitamin E) [5]. In vivo, tumor growth of xCT-KO cells xenografts was delayed but not suppressed. Dr Vucetic explained that xCT plays its fundamental role in cyst(e)ine import exclusively or almost exclusively in an oxidized form (*in vitro* conditions), and that *in vivo*, cysteine-reduced form can come from different sources. Interestingly, this work demonstrated that neighboring fibroblasts secrete small "rescue agents" for the xCT-KO tumors allowing the complete prevention of ferroptosis [6].

In conclusion, Milica Vucetic and collaborators show that the cysteine transporter xCT is a potential target for treating pancreatic cancer, nevertheless their findings suggest that xCT inhibition has to be achieved systematically in order to avoid cell-to-cell interplay.

Redox regulation has been proposed to control various aspects of carcinogenesis, cancer cell growth, metabolism, migration, invasion, and metastasis. As cancer has many faces, the role of redox control in different cancers and in the numerous cancer-related processes often points to different directions. Holly Brunton (CRUK Beatson, United Kingdom) in a selected talk focused on Selenocysteine, a cysteine analogue that is more efficient at 'mopping up' free radicals. Selenocysteine has a structure similar to cysteine, but with an atom of selenium replace the sulfur. Proteins which contain a selenocysteine residue are called selenoproteins. Holly Brunton and collaborators interrogate the transcriptome and metabolome of 48 novel pancreatic cancer patient derived cell lines (PDCLs) to define mechanisms of resistance to GSK3β inhibition using a GSK3β inhibitor (9-ING-41) with antitumor activity. They observed that redox metabolites increase following targeted therapy in PDAC. RNA-seq analysis of resistant PDCLs showed that selenocysteine metabolism is significantly altered in resistant PDCLs. After prolonged inhibitor treatment, they observed an increase in *PSTK* and *SEPSECS* expression, encoding the two kinases implicated in selenoprotein translation. When cells are

grown in the absence of selenium, translation of selenoproteins terminates at the UGA stop codon, resulting in a truncated non-functional enzyme, suggesting that to increase translation of ROS-clearing proteins, a cell will require an increased supply of selenium. In conclusion, Brunton and collaborators suggest that targeting selenoprotein translation will reduce the cellular capacity to manage ROS and resensitize PDAC tumors to targeted therapy.

Pierre Sonveaux (UC Louvain, Belgium) hypothesized that cancer metastasis is driven, at least partly, by metabolic alterations. Although aerobic glycolysis is known to promote metastasis, Sonveaux and collaborators identified a different switch primarily affecting mitochondria. This switch involves overload of the electron transport chain (ETC) with preserved mitochondrial functions but increased mitochondrial superoxide production [7]. Experimentally, Sonveaux successfully selected super-invasive (*in vitro*) and super-metastatic (*in vivo*) cells. Interestingly, the *in vitro* selection did not induce a glycolytic switch (on contrary to the well-recognized avidity for glucose of metastatic tumors) or lactate production. Moreover, the super-metastatic tumor cells acquired giant mitochondria with higher cristae number and a mitochondrial overload. Of major importance, this mitochondrial overload correlates with tumor metastasis and consistently, moderate Complex I inhibition promoted mitochondrial ROS-dependent tumor cell migration. Moreover, mitochondria controlled spontaneous metastatic dissemination of B16F10 mouse melanoma and triple-negative MDA-MB-231 human breast cancer (TNBC) cells in xenograft models. Finally, this group introduced “mitoQ”, a compound inhibiting TNBC recurrence and spontaneous metastasis in mice, placing it as a potential promising drug to prevent metastasis.

Mitochondrial respiration (oxidative phosphorylation, OXPHOS) is an emerging target in currently refractory cancers such PDAC. However, the variability of energetic metabolic adaptations among PDAC patients has not been fully investigated. In a selected talk, Nadine Abdel Hadi (Marseille, France) demonstrated that OXPHOS rates are highly heterogeneous in

PDAC patients, and that high OXPHOS tumors are enriched in mitochondrial respiratory complex I at both the protein and mRNA levels. Furthermore, phenformin, a mitochondrial complex I inhibitor, has a synergistic effect when combined with Gemcitabine in high-OXPHOS tumors in orthotopic mouse xenograft models [8]. Abdel Hadi showed that PDAC cells depend on fatty acids as an energy source to feed mitochondrial respiration and identified Fatty Acid Oxidation (FAO) as a novel vulnerability of this highly aggressive cancer. Then, she asked whether Perhexiline, a FAO inhibitor, can amplify the antitumoral activity of Gemcitabine in PDAC tumors. Combining Gemcitabine and Perhexiline induced complete tumor regression in PDAC xenografts, although relapse was still observed after long term treatment due to metabolic adaptations of persistent cells. This work showed that relapsed PDAC tumors result from the proliferation of persistent cells (also called residual) which survived after therapy-induced regression, through the establishment of metabolic adaptations in particular modifications of redox metabolism. This supports the strategy of treating the minimal residual pancreatic disease with drugs that increase ROS levels to reach a ROS concentration promoting cell death to prevent the relapse.

SESSION II: Redox and hypoxia

The meeting hosted the Nobel prize laureate Sir Peter Ratcliffe (Ludwig Institute for Cancer Research, University of Oxford and the Francis Crick Institute, London, United Kingdom) who gave a keynote lecture on our understanding on oxygen sensing by metazoan and its implication in cancer and therapeutics. The hypoxia-inducible factor (HIF) transcription factor plays a central role in hypoxia [9]. Under normoxic conditions (21% O_2), the alpha subunit of HIF (HIFα) is hydroxylated at conserved proline residues by 2-oxoglutarate-dependent oxygenase, HIF prolyl-hydroxylases (PHDs), allowing its recognition and ubiquitination by the E3 ubiquitin ligase VHL, which labels it for rapid degradation by the proteasome. Under low

oxygen conditions, or in cells lacking functional pVHL, HIFα accumulates, dimerizes with an HIFβ family member, translocates to the nucleus, and transcriptionally regulates genes involved in erythropoiesis, angiogenesis, autophagy, and energy metabolism. Genomic analyses revealed at least one isoform of each component of the HIF-PHD-VHL triad in each animal species. Most primitive invertebrates possess a single set of proteins, with multiple isoforms arising through gene duplication events at the basis of vertebrate evolution. Substrate repertoire analysis revealed that PHDs mainly target HIF [10]. It becomes clear that all four eucaryotic kingdoms use different types of protein oxidation coupled to proteolysis and to oxygen levels [11]. For example, oxygen sensing system in plants is based on cystein oxidation [12]. Ratcliff *et al.* identified a protein ADO (cysteamine (2-aminoethanethiol) dioxygenase) in human cells that is the human ortholog of the plant cystein oxidase mediating responses to hypoxia [13]. In cancer, oxygen-sensing pathways are dysregulated, both by oncogenic mutations and by micro-environmental hypoxic conditions that occur in most solid tumors. In renal cell carcinomas, constitutive activation of HIF results from inactivating mutations of the VHL tumor suppressor, and this leads to stabilization of high levels of HIF even in well-oxygenated cells. In this situation, the switch of interconnected hypoxia pathways is predicted to generate anti-tumorigenic as well as pro-tumorigenic effects [14]. In this way, un-physiological activation of interconnected pathways in cancer, as exemplified by HIF, promote selective mechanisms that drive escape mechanisms and fuel the flexibility and heterogeneity to enable many tumors to avoid chemotherapeutic eradication [15].

Hypoxia resistance in tumors was further discussed by Amato Giaccia (Oxford Institute for Radiation Oncology, University of Oxford, United Kingdom) who investigated the implication of hypoxia in breast cancer resistance to Poly (ADP-ribose) polymerase (PARP) inhibitors. The breast cancer–associated genes 1 and 2 (*BRCA1*, *BRCA2)* proteins play a central role in the repair of double-strand DNA breaks *via* homologous recombination (HR). Because cells that

are deficient in *BRCA1* or *BRCA2* display impaired HR and an inability to repair defective chromosomes [16]. This defect makes BRCA-deficient tumors good candidates for therapies based on PARP inhibitors. PARP binds to damaged DNA through its zinc finger domain and helps the altered structure to become more organized and accessible to critical co-factors that are essential for its activity. PARP inhibitors (PARPi) trap PARP proteins on DNA and block their catalytic action. This interferes with replication, causing cell death preferentially in cancer cells due to their high replication rate. It was showed that severe hypoxia (<0.5% O_2) compromises HR and synergizes with PARPi in HR proficient cells causing cell death [17]. However, Giaccia's group found that in moderate hypoxia (2% O_2) HR-deficient as well as HR-proficient tumors are resistant to PARPi therapy [18]. This was due neither to a loss of PARP activity nor to a non-response to PARPi, suggesting that drug resistance in these tumors is linked to hypoxia. In contrast, PARPi in combination with inhibitors of alternative nonhomologous end joining repair (alt-NHEJ) enzymes resulted in increased cytotoxic double-strain breaks and cell death under normoxic conditions. Giaccia's group raised the hypothesis that the mechanism of resistance was associated with reduction of reactive oxygen species (ROS)-induced DNA damage in hypoxia. Their work demonstrates that synthetic lethality and PARPi efficacy can be restored in hypoxic HR-deficient cancer cells resistant to PARPi, through the addition of a ROS-generating hypoxia-activated cytotoxin such as tirapazamine (TPZ). Therefore, Giaccia's group proposes the use of TPZ and other hypoxic cytotoxins in combination with PARPi-based therapy in future clinical trials for treatment of hypoxic tumors that are unresponsive to PARPi therapy alone [19].

The critical role of HIF-1 for the evolution of prostatic pre-cancer lesions into fully malignant tumors was discussed by Mohamed Abu El Maaty (Institut de Génétique et de Biologie Moléculaire et Cellulaire University of Strasbourg, France). He utilized genetically engineered mouse models in which the tumor suppressor Pten is selectively deleted (Pten(i)pe-/- mice) in

prostate using a Cre driver based on the probasin promoter. Single cell level analyses revealed the presence of immunosuppressive myeloid-derived cells, T lymphocytes and stromal fibroblastic but also luminal C cells expressing stem cells markers. Activation of HIFα signaling during disease progression was also observed. To study the role of HIF1α in prostate carcinogenesis, Abu El Maaty then generated Pten/Hifα(i)pe-/- mice in which both Pten and HiFα were selectively invalidated in prostate luminal epithelial cells at adulthood. This model revealed that HIF1α promotes progression of prostatic intraepithelial neoplasia (PIN), as well as the development of poorly differentiated tumors. Single-cell characterization of prostates isolated from Pten/Hifα(i)pe-/- mice at 3 months of age demonstrated the downregulation of glucose-metabolizing networks and modulation of senescence-associated secretory phenotype in luminal cells. These effects led to a stimulation of immune surveillance in prostate, characterized by a reduction in the infiltration of myeloid-derived suppressor cells and increased levels of CD8+T-lymphocytes and natural killer cells, leading to apoptosis. Furthermore, expression of the pluripotency and plasticity factors Sox2 and Ezh2 was lower in Pten/ HiFα(i)pe-/- cells compared to Pten(i)pe-/- cells, and epithelial cells with HIFα ablation displayed reduced organoid formation capacity. Also, expression of several chemokines (CXCL2, CXCL5 and CXCL17) with the stimulating cytokine IL12 a functional stimulator of CD8+ T lymphocytes were detected. Altogether, these results confirmed that HIFα signaling is a key driver of PIN progression by promoting cell-intrinsic and-extrinsic pro-tumoral pathways. The relation between hypoxia and its role in promoting stemness in cancer cells was finally discussed by Persio Dello Sbarba (Dipartimento di Scienze Biomediche Sperimentali, University of Florence, Italy) who presented the role of hypoxia in the selection and maintenance of leukemic stem Cells (LSCs). Chronic myeloid leukemia (CML) is a hematopoietic stem cell (HSC)-driven neoplasia characterized by expression of the constitutively active tyrosine kinase BCR/Abl. Dello Sbarba's group found that incubating

CML cells in low oxygen decreases BCR/Abl protein, but not BCR/Abl mRNA levels, in a time-dependent manner. Under these conditions, stem cell potential is maintained independently of BCR/Abl signaling and a LSC subset is still present together with a remaining leukemic gene signature. Because of the decreased expression of their molecular target, LSC are refractory to tyrosine kinase inhibitors (TKi) which are used to target BCR/Abl in CML therapies. However, in this heterogeneous population, a subset of cells maintaining BCR/Abl expression (referred to as "BCR/Abl-positive") display LSC properties. In contrast, CML cells incubated in standard atmosphere (20% O_2) with a TKi (Imatinib) exhibit marked reduced viability [20]. Based on these results, Dello Sbarba proposed that BCR/Abl-positive LSC ("LSC in progenitor cells") drive the expansion of leukemic cell populations, whereas BCR/Abl-negative LSC ("LSC in stem cells") are responsible for the long-term maintenance of therapy-resistant cells during the minimal residual disease (MRD) phase of CML. An initial characterization of metabolic mechanisms driving BCR/Abl protein suppression showed that it occurs when the glucose concentration is closed to complete exhaustion but reappeared when glucose concentration increased. Thus, Dello Sbarba's group revealed a new mechanism of resistance of CML cells to TKi based on the reversible expression of the BCR/Abl protein depending on local substrate availability in low oxygen stem cell niche [21]. In SCN zones where glucose is available BCR/Abl expression would predispose CML cells to clonal expansion, whereas zones under glucose shortage would host cells adapted to persist independently of BCR/Abl signaling and thus representing a reservoir of treatment-resistant MRD. According to this model, a relapse of disease would occur when BCR/Abl-negative LSC adapted to energy shortage, following the establishment of permissive conditions (restored glucose supply), are induced to turn into or generate BCR/Abl-positive LSC capable to sustain clonal expansion [22].

SESSION III: Metabolic profiling and epigenetic control

The molecular consequences of cancer cell metabolic reprogramming are not yet fully understood. It is well recognized that some of the metabolites abnormally produced or consumed by cancer cells serve as essential cofactors by DNA or RNA modifying enzymes. This session focused on studies aiming at further understanding the complex connections between metabolism and the epigenome or the epitranscriptome. The intricate links between metabolism and DNA methylation or histone modifications have been thoroughly investigated in the past decades and several speakers of the meeting, including Dr. Judith Favier (INSERM, Paris Univ.), Dr. Robert Schneider (Institute of functional epigenetics, Munich Univ.) and Assia Tiamou (GIGA, Liege Univ.) contributed to this field through their original findings.

Dr. Favier's laboratory is trying to understand the mechanisms by which mutations in genes encoding subunits of the succinate dehydrogrenase (SDH) promote pheochromocytoma and paraganglioma (PPGL) development, two rare neuroendocrine tumors arising in the adrenal medulla and the parasympathetic or sympathetic nervous systems, respectively. The SDH enzyme is a multisubunit complex of the tricarboxylic acid (TCA) cycle that converts succinate to fumarate. A mutation of any subunit of the SDH complex leads to the accumulation of succinate, an oncometabolite which inhibits 2-oxoglutarate (2-OG) -dependent dioxygenases. Succinate-mediated inhibition of several of these enzymes has been proposed to contribute to oncogenesis, including prolyl-hydroxylases (PHDs) that are necessary for the degradation of the hypoxia-inducible factors (HIFs), the ten-eleven translocation (TET) enzymes that hydroxylate 5-methylcytosine into 5-hydroxymethylcytosine (5hmC), as well as members of the Jumonji (JmjC) family of lysine demethylases. These defects were proposed to lead to a pseudohypoxic state and a hypermethylation phenotype observed in the most aggressive forms of SDH-deficient tumours. However, their relative contribution to cancer progression was still debated. Another unsolved question in that field relates to the much worse prognosis of patients

with SDHB-mutated tumors compared to those harbouring mutations in other subunits of the SDH complex. Consistent with these clinical observations, SDHB-mutated cells are more invasive and display features linked to the epithelial-to-mesenchymal transition (EMT). Using genetic approaches, this laboratory elegantly demonstrated that inhibition of the TET1/2 enzymes was sufficient to recapitulate the DNA hypermethylation phenotype of SDHB-mutated cells but failed to increase their invasive capacity. Strikingly, culturing TET1/2-deficient, but not control cells in mild hypoxic conditions induced a mesenchymal-like phenotype comparable to that of SDHB deficient cells. These data support a model where the epigenetic reprogramming induced by TET1/2 inhibition primes these cells to HIF2-mediated induction of an EMT, a process which increases their metastatic potential. Interestingly, Favier's laboratory also recently identified a new mechanism involved in the regulation of iron metabolism which participates in the reprogramming of SDHB-deficient cells. SDHB is an iron-sulphur cluster containing protein and its inhibition leads to decreased mitochondrial Fe^{+2} levels and to increased mitochondrial ROS production. This defect in iron metabolism sensitizes SDHB, but not SDHD-mutant cells to pro-oxidant molecules, raising potential interesting clinical perspectives to design new therapeutic strategies to target specifically SDHB-mutated tumors [23].

A selected talk was presented by Assia Thiamou from Liège University in Belgium. She identified a link between methylglyoxal (MG), a highly reactive by-product of glycolysis, and DNA methylation. She illustrated another interesting example linking the metabolic reprogramming of cancer cells to their epigenome. In triple negative breast cancer (TNBC) cells, shRNA-mediated knockdown of GLO1 or GLO2, two enzymes involved in the detoxification of MG, or MG treatment, increased CpG methylation through the upregulation of the DNMT3A and DNMT3B DNA methyltransferases. Consistent with the importance of the DNA hypermethylation phenotype in the transcription of cancer-related genes in these

TNBC cells, treatment with the demethylating agent 5AZA reverted the effects of MG accumulation on the transcription of tumour suppressor genes and on their migratory capacity. Altogether, these unpublished data link glycolysis to MG stress and DNA hypermethylation, highlighting an additional mechanism by which the deregulation of this key metabolic pathway contributes to the aggressiveness of cancer cells.

Histone post-translational modifications are also important integrators of signals originating from metabolic changes. Enzymes which use the metabolites S-Adenosyl-Methionine (SAM) and AcetylCoenzymeA (AcCoA) as essential cofactors (methyl- and acetyl- transferases, respectively), mediate methylation and acetylation, the two most studied modifications of histone tails. Robert Schneider's lab (Institute of Functional Epigenetics, Munich, Germany) made important findings to show that these two modifications of histones are likely to represent just the tip of the iceberg and that many lysine residues of histone tails are also targets for other types of acylations including butyrylation and propionylation which contribute to transcriptional activation. These modifications are intrinsically linked to the metabolic status of these cells since their addition requires propionyl-CoEnzymeA and butyryl-Coenzyme A as cofactors, two products of lipid and amino-acid catabolism. We are just beginning to grasp the functions of these different acylations, but they raise important questions regarding the mechanisms by which the availability of specific nutrients can influence gene expression.

These examples illustrate the ever-growing notion that metabolic changes can alter gene expression through modifications of DNA or histones, however, much less is known about potential connections between metabolites and RNA modifications. An original finding by the Schneider lab was presented at this meeting, linking two methyltransferases, METTL5 and METTL6 (Methyltransferase Like 5 and 6) to RNA methylation. Strikingly, METTL5 failed to show any DNA methyltransferase activity *in vitro* but efficiently methylates total RNA. Mass-spectrometry analyses showed that METTL5 catalyses the generation of N6-methyladenosine

(m^6A) of the 18S ribosomal RNA at position 1832, a modification influencing protein translation in embryonic stem (ES) cells and thereby their pluripotency. *Mettl5* knock-out (KO) mice display phenotypes that are reminiscent of some of the clinical symptoms observed in patients harbouring *Mettl5* mutations, including intellectual and locomotor deficiencies. The Schneider laboratory also studies METTL6, another RNA methyltransferase that was initially identified in a genetic screen aiming at identifying methyltransferases influencing tumour growth. METTL6 catalyses 3-Methylcytidine (m3C) modification of a subset of serine tRNAs at position C32, thereby influencing codon specific translation. Interestingly, *Mettl6* KO mice display metabolic phenotypes and liver defects, highlighting a previously unknown role for this RNA modification in glucose metabolism.

Another interesting illustration of the close links between RNA biology and metabolism came from a selected talk presented by Yvan Martineau (CRCT, University of Toulouse). He used a translatome approach to classify pancreatic cancers and identified an interesting subgroup of PDACs characterized by the induction of the Integrated Stress Response (ISR) and a higher translation rate of *ATF4*, a key mediator of this cellular pathway which is activated in response to several stress types including decreased amino-acid availability. This group showed that the induction of the ISR response in these cancer cells reflects their inability to express genes implicated in de novo serine synthesis and the transulfuration pathway despite high levels of ATF4, thereby leading to high dependency on exogenous serine and cysteine [24].

These few examples are very illustrative of the complexity of the connections between metabolism and the epigenome and the epitranscriptome. Looking deeper into those links is likely to provide new anti-cancer strategies.

Technological breakthroughs are of the utmost importance in order to shed light on the mechanisms driving the metabolic reprogramming of cancer cells. Single cell approaches to study metabolism are still in their infancy and therefore the work performed by Rafael Jose

Argüello's team (CIML, Marseille, France) is of major interest. This team developed SCENITH, a flow cytometry-based assay aiming at analyzing energetic metabolism at the single cell level [25]. SCENITH allows for the study of metabolic responses in multiple cell types in parallel by flow cytometry and is suitable to perform metabolic studies ex vivo, particularly in rare cells in whole blood/tissue samples, avoiding metabolic biases introduced by culture media. SCENITH is an original and powerful method to reveal metabolic heterogeneity in complex systems and to link metabolic features to other molecular information (such as epigenetic marks), even in rare cell subpopulations. Such an approach is needed to further characterize the complexity of metabolic changes in cancer which is likely to improve patient stratification.

SESSION IV: Signaling, fueling and metabolism in cancer

In the context of cancer cells, the duality of the immune system is of utmost significance, as it contributes to both the development and diminution of malignant cells. Over the course of the last decade, advances in immunotherapy have provided a marked improvement in cancer treatment. In this regard, Maya Saleh and her team (Immonucept, University of Bordeaux) focus on the impact of metabolic alteration of the immune system during auto-immune disease and cancer. To this end, they performed a single-cell approach to characterize liver immune cells during the development of hepatocellular carcinoma (HCC) [26]. Specifically, they identified two genes, O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA) involved into the polarization of tumor-associated macrophage (TAM) (Fig. 1). These genes encode two rate-limiting enzymes implicated in hexosamine biosynthetic pathway (HBP). This branch of glycolysis produces UDP-GlcNAc, a substrate for protein glycosylation, that acts as a novel determinant of macrophage response. The absence of OGT and OGA significantly decreased tumor growth in glioma *in vivo*. Dr. Saleh highlighted a novel aspect of the HBP pathway as a

promising therapeutic target for the reduction of inflammation and the reactivation of anti-tumor immunity.

Lipotoxicity is the consequence of excessive accumulation of lipids in cells and organelles which causes endoplasmic reticulum and oxidative stress, thus leading to cell death. Dr. Alicia Kowaltowski's work (Institute of Chemistry, Brazil) aims at discovering the early bioenergetic results of lipid overload in hepatic cells [27]. The treatment of cells with palmitate (the most abundant saturated fatty acid in human serum) caused fragmentation in mitochondrial morphology. Moreover, the accumulation of palmitate caused a glycolytic shift (Pasteur effect), resulting in the increase in glycolytic flux and ATP production. This also promoted the production of reactive oxygen species (ROS) at different sites of the electron transport chain (ETC), leading to a redox imbalance. Selective ETCIII inhibitors (S1QEL and S3QEL) demonstrated that increasing ROS (H_2O_2) contributes to the metabolic rewiring induced by palmitate (not oleate) accumulation. Such observations require further analysis, especially regarding the notion that lipotoxicity contributes to the regulation of glycolysis and redox imbalance in hepatic cells.

Metabolic reprogramming is a major determinant of cancer development and is defined as a hallmark of cancer by Hanahan and Weinberg. [28]. During recent years, the identification of key metabolite candidates for survival has become a potential therapeutic approach in cancer. In the liver, transcription factor Carbohydrate Responsive Element Binding Protein (ChREBP) is the main mediator in glucose sensing and utilization. It plays a central role in the modulation of glucose action among glycolytic, pentose phosphate and lipogenic gene expression. Interestingly, ChREBP is over expressed in Hepatocarcinoma (HCC) and is also associated with poor prognosis, thus posing as a good candidate for study in the context of HCC. Emmanuel Benichou (Institute Cochin, Paris) showed that overexpression of ChREBP in murine liver using a transposon system promotes initiation and development of HCC and

recapitulates the course of the human disease in mice. This overexpression is associated with a rewiring of glutamine and glucose metabolism, which leads to enhance *de novo* nucleotide biosynthesis and sustainable growth *[unpublished]*. These results suggest that the pharmacological inhibition of ChREBP represents a promising therapeutic approach in the treatment of HCC (Fig. 1).

In response to stress conditions such as chemical toxicity, nutritional and oxidative stress, or mechanical (shear) stress, intrinsic pathways of autophagy can be activated in kidney epithelial cells (KEC). Such adaptive and dynamic mechanisms are characterized by selective degradation of cellular components and by the recycling of macromolecules. Etienne Morel (Institute Necker, Paris) demonstrated the implication of shear stress during the specific dialogue between primary cilium and the autophagic machinery of KECs [29]. The fluid flow sensing capabilities of primary cilium favors mitochondrial biogenesis through the increased expression of mitochondrial master regulators such as Mitochondrial transcription factor A (TFAM) and PGC1α. This leads to a profound metabolic reprogramming, which includes an increase in oxygen consumption and total ATP production, essential to fully differentiate KECs. More specifically, fluid flow promotes lipophagy, a specialized autophagic program that degrades lipid droplets, thus contributing to the production of free fatty acids providing mitochondrial substrates to fuel mitochondria and generate ATP through β-oxidation. This specific metabolic interaction between shear stress and autophagy potentially explains how primary cilia use lipophagy to fuel mitochondria and enable the proper translation of mechanical forces.

PDAC, the most common form of pancreatic cancer, is an extremely lethal disease due to late diagnosis, aggressiveness, and lack of effective therapies. In this context, Beatriz Parejo-Alonso (IIS Aragon, Zaragoza) discussed the role of Peroxisome Proliferator-activated Receptor Delta (PPARD) in the MYC/PGC-1α-dependent metabolic phenotype of PDAC cells. This molecular cascade controls stemness and invasiveness of PDAC, as well as the implication of TAM

microenvironmental signals in this pathway [30]. This group demonstrated that PPARD is upregulated after treatment with EMT inducers or TAM-conditioned medium. Interestingly, pharmacological activation of PPARD induces invasion associated with an increase in EMT-related gene expression. The metabolic switch induced by PPARD and MYC/PGC-1α is characterized by a decrease in oxygen consumption and an increase in glycolysis. Conversely, PPARD inhibition increases the invasive capacity of highly metastatic cells and enhances mitochondrial metabolism (the increase of oxygen consumption and ATP-linked respiration) associated with a decrease in glycolysis metabolism (the decrease of extracellular acidification). This research highlights the pro-metastatic role of PPARD in PDAC and proposes a potential therapeutic approach for treating cancers with high PPARD.

A multitude of studies clearly reveal that obesity can lead to increased cancer risk. Studies aiming at investigating the impact of diets on cancer progression became a field of active research in recent years. In this context, Dr. Karen Vousden (The Francis Crick Institute, London) discussed some of their pioneer work in which they addressed how specific diets influence cancer development and therapeutic responses. Her work initially focused on *TP53*, the most frequently mutated tumor suppressor in human cancer. Over the past years, Dr. Vousden and colleagues demonstrated that p53 plays a protective role when cells face a nutrient challenge. During this meeting, she presented an elegant *in vivo* reporter system of p53 transcriptional activity that they used to show that p53 promotes normal liver function by limiting stress in hepatocytes when animals are fed with a high fat diet. An important aspect of p53-associated metabolic functions relates to its ability to sustain serine/glycine metabolism. These two non-essential amino acids, which can be either taken up from the environment or newly synthesized through the serine synthesis pathway, play important roles in many anabolic reactions including redox homeostasis as well as protein, nucleotide and lipid biosynthesis. Several groups, including Dr. Vousden's team previously showed that a functional p53 pathway

is essential for cells to stimulate de novo serine synthesis in conditions when exogenous serine and glycine become limiting. In this meeting, she showed some of their recent data indicating that pharmacological inhibition of phosphoglycerate dehydrogenase (PHGDH), a rate-limiting enzyme in this anabolic pathway, impinges on cancer growth when combined with a serine and glycine -deprived diet [31]. The effect involves a strong cooperative effect on the one carbon cycle, a key metabolic pathway involved in purine synthesis. This evidence supports the notion that dietary modulation is a promising strategy to enhance the efficiency of anti-cancer therapeutics.

General public session of nutrition and cancer

Following the 2-day scientific meeting, an online event for patients and the general public, focusing on cancer and nutrition, was organised. Since 2017, the SIRIC BRIO (Bordeaux integrated cancer research site) has developed an expertise in mixing scientific discussions and comedian performances for general public events. On this occasion, it teamed with the national network on nutrition and cancer (NACRe) and the association “soins de support” to organize an online event in French.

The concept included a comedian couple (acting as a patient and his wife) who reacted and played a scene after each scientific presentation, as if they were watching a television broadcast from their house. The content of their performance had been prepared through interviews of the scientists and was closely inspired by their messages, while the added off-the-wall humour had been previously tested with BRIO’s patient and carer collective. This allowed different perspectives on the issues, prevented the tone from being too serious, included a change of rhythm and overall reinforced the messages either by repetition or by opposition.

The three-hour meeting started by a short explanation of what is cancer metabolism research and what the scientific meeting had been about. It then covered, in a very accessible manner,

the impact of nutrition during and after treatment, restrictive diets, undernourishment and the special issue of older patients. One presentation also commented on the commonly heard messages on nutrition and cancer. The overall idea was to insist on what is proven and what is not, what could potentially be harmful and where to get reliable information. Interestingly, after each presentation the actors highlighted the important role of the family members accompanying the patients. Finally, the eight scientists answered many questions from the public in a dedicated session.

This successful experience should encourage us to offer more opportunities of interactions between scientists and the public. When academic meetings are organised, a public event (in the national language) should be considered. This is particularly important on health issues for which the information discussed helps empower people in their personal life. Despite the lack of direct interaction, webinars are useful to reach a wide audience, and audacious formats, where trusted scientific content is presented with a twist, can still be imagined. Out of the context of a pandemic, the event would ideally be co-created with patients and carers, as done previously for BRIO's public events.

Conclusion

Many aspects of cancer metabolism were covered during this meeting. The speakers highlighted some of the newest findings showing how metabolism plays an essential role in the initiation and progression of carcinogenesis, and how it modulates the response to different therapies. Cancer cells are dependent on many metabolic pathways and this dependence represents interesting targets. A better understanding of the complex signaling pathways that modulate metabolism will help designing new therapies that may synergize with standard treatments. Moreover, diet modulation can alter cancer growth by limiting the metabolic plasticity of cancer

cells. Collectively, these presentations confirm the utility of novel therapeutics targeting cellular metabolism to improve patient outcome.

Authors contribution: NAH, EB, AL, LL, AR, GRC and NC wrote the manuscript, AC, LLC, NMM, JER, SR, JES, SV, MVL, RR and FB read and corrected the manuscript

Acknowledgements We thank La Ligue Nationale contre le cancer, the ARC foundation and the Société Française du Cancer for their support. The meeting was sponsored by The Canceropole Sud-Ouest, The Region Nouvelle-Aquitaine and the Université of Bordeaux. The public session on nutrition and cancer was organized in collaboration with the French network on nutrition and cancer (NACRe), the French association “soins de support” and the SIRIC BRIO (Bordeaux Recherche Intégrée en Oncologie), the Bordeaux integrated cancer research site. We would like to address special thanks to Karine Marendziak, Bernard Srour and the team of Comnco.

REFERENCES

1. Chouchani, E. T., Pell, V. R., Gaude, E., Aksentijević, D., Sundier, S. Y., Robb, E. L., Logan, A., Nadtochiy, S. M., Ord, E. N. J., Smith, A. C., Eyassu, F., Shirley, R., Hu, C. H., Dare, A. J., James, A. M., Rogatti, S., Hartley, R. C., Eaton, S., Costa, A. S. H., Brookes, P. S., Davidson, S. M., Duchen, M. R., Saeb-Parsy, K., Shattock, M. J., Robinson, A. J., Work, L. M., Frezza, C., Krieg, T. & Murphy, M. P. (2014) Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS, *Nature.* **515**, 431-435.

2. Chouchani, E. T., Pell, V. R., James, A. M., Work, L. M., Saeb-Parsy, K., Frezza, C., Krieg, T. & Murphy, M. P. (2016) A Unifying Mechanism for Mitochondrial Superoxide Production during Ischemia-Reperfusion Injury, *Cell metabolism.* **23**, 254-63.

3. Mills, E. L., Kelly, B., Logan, A., Costa, A. S. H., Varma, M., Bryant, C. E., Tourlomousis, P., Däbritz, J. H. M., Gottlieb, E., Latorre, I., Corr, S. C., McManus, G., Ryan, D., Jacobs, H. T., Szibor, M., Xavier, R. J., Braun, T., Frezza, C., Murphy, M. P. & O'Neill, L. A. (2016) Succinate Dehydrogenase Supports Metabolic Repurposing of Mitochondria to Drive Inflammatory Macrophages, *Cell.* **167**, 457-470.e13.

4. Yin, Z., Burger, N., Kula-Alwar, D., Aksentijević, D., Bridges, H. R., Prag, H. A., Grba, D. N., Viscomi, C., James, A. M., Mottahedin, A., Krieg, T., Murphy, M. P. & Hirst, J. (2021) Structural basis for a complex I mutation that blocks pathological ROS production, *Nature communications.* **12**, 707.

5. Daher, B., Parks, S. K., Durivault, J., Cormerais, Y., Baidarjad, H., Tambutte, E., Pouysségur, J. & Vučetić, M. (2019) Genetic Ablation of the Cystine Transporter xCT in PDAC Cells Inhibits mTORC1, Growth, Survival, and Tumor Formation via Nutrient and Oxidative Stresses, *Cancer research.* **79**, 3877-3890.

6. Meira, W., Daher, B., Parks, S. K., Cormerais, Y., Durivault, J., Tambutte, E., Pouyssegur, J. & Vučetić, M. (2021) A Cystine-Cysteine Intercellular Shuttle Prevents Ferroptosis in xCT(KO) Pancreatic Ductal Adenocarcinoma Cells, *Cancers.* **13**.

7. Porporato, P. E., Payen, V. L., Pérez-Escuredo, J., De Saedeleer, C. J., Danhier, P., Copetti, T., Dhup, S., Tardy, M., Vazeille, T., Bouzin, C., Feron, O., Michiels, C., Gallez, B. & Sonveaux, P. (2014) A mitochondrial switch promotes tumor metastasis, *Cell reports.* **8**, 754-66.

8. Masoud, R., Reyes-Castellanos, G., Lac, S., Garcia, J., Dou, S., Shintu, L., Abdel Hadi, N., Gicquel, T., El Kaoutari, A., Diémé, B., Tranchida, F., Cormareche, L., Borge, L., Gayet, O., Pasquier, E., Dusetti, N., Iovanna, J. & Carrier, A. (2020) Targeting Mitochondrial Complex I Overcomes Chemoresistance in High OXPHOS Pancreatic Cancer, *Cell reports Medicine.* **1**, 100143.

9. Ratcliffe, P. J., O'Rourke, J. F., Maxwell, P. H. & Pugh, C. W. (1998) Oxygen sensing, hypoxia-inducible factor-1 and the regulation of mammalian gene expression, *The Journal of experimental biology.* **201**, 1153-62.

10. Chowdhury, R., Leung, I. K., Tian, Y. M., Abboud, M. I., Ge, W., Domene, C., Cantrelle, F. X., Landrieu, I., Hardy, A. P., Pugh, C. W., Ratcliffe, P. J., Claridge, T. D. & Schofield, C. J. (2016) Structural basis for oxygen degradation domain selectivity of the HIF prolyl hydroxylases, *Nature communications.* **7**, 12673.

11. Kaelin, W. G., Jr. & Ratcliffe, P. J. (2008) Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway, *Molecular cell.* **30**, 393-402.

12. Loreti, E., van Veen, H. & Perata, P. (2016) Plant responses to flooding stress, *Current opinion in plant biology.* **33**, 64-71.

13. Masson, N., Keeley, T. P., Giuntoli, B., White, M. D., Puerta, M. L., Perata, P., Hopkinson, R. J., Flashman, E., Licausi, F. & Ratcliffe, P. J. (2019) Conserved N-terminal cysteine

dioxygenases transduce responses to hypoxia in animals and plants, *Science (New York, NY).* **365**, 65-69.

14. Wykoff, C. C., Pugh, C. W., Maxwell, P. H., Harris, A. L. & Ratcliffe, P. J. (2000) Identification of novel hypoxia dependent and independent target genes of the von Hippel-Lindau (VHL) tumour suppressor by mRNA differential expression profiling, *Oncogene.* **19**, 6297-305.

15. Pugh, C. W. & Ratcliffe, P. J. (2017) New horizons in hypoxia signaling pathways, *Experimental cell research.* **356**, 116-121.

16. Farmer, H., McCabe, N., Lord, C. J., Tutt, A. N., Johnson, D. A., Richardson, T. B., Santarosa, M., Dillon, K. J., Hickson, I., Knights, C., Martin, N. M., Jackson, S. P., Smith, G. C. & Ashworth, A. (2005) Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy, *Nature.* **434**, 917-21.

17. Chan, N., Pires, I. M., Bencokova, Z., Coackley, C., Luoto, K. R., Bhogal, N., Lakshman, M., Gottipati, P., Oliver, F. J., Helleday, T., Hammond, E. M. & Bristow, R. G. (2010) Contextual synthetic lethality of cancer cell kill based on the tumor microenvironment, *Cancer research.* **70**, 8045-54.

18. Chiappa, M., Guffanti, F., Bertoni, F., Colombo, I. & Damia, G. (2021) Overcoming PARPi resistance: Preclinical and clinical evidence in ovarian cancer, *Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy.* **55**, 100744.

19. Mehibel, M., Xu, Y., Li, C. G., Moon, E. J., Thakkar, K. N., Diep, A. N., Kim, R. K., Bloomstein, J. D., Xiao, Y., Bacal, J., Saldivar, J. C., Le, Q. T., Cimprich, K. A., Rankin, E. B. & Giaccia, A. J. (2021) Eliminating hypoxic tumor cells improves response to PARP inhibitors in homologous recombination-deficient cancer models, *The Journal of clinical investigation.* **131**.

20. Tusa, I., Cheloni, G., Poteti, M., Silvano, A., Tubita, A., Lombardi, Z., Gozzini, A., Caporale, R., Scappini, B., Dello Sbarba, P. & Rovida, E. (2020) In Vitro Comparison of the Effects of Imatinib and Ponatinib on Chronic Myeloid Leukemia Progenitor/Stem Cell Features, *Targeted oncology.* **15**, 659-671.

21. Giuntoli, S., Tanturli, M., Di Gesualdo, F., Barbetti, V., Rovida, E. & Dello Sbarba, P. (2011) Glucose availability in hypoxia regulates the selection of chronic myeloid leukemia progenitor subsets with different resistance to imatinib-mesylate, *Haematologica.* **96**, 204-12.

22. Bono, S., Lulli, M., D'Agostino, V. G., Di Gesualdo, F., Loffredo, R., Cipolleschi, M. G., Provenzani, A., Rovida, E. & Dello Sbarba, P. (2016) Different BCR/Abl protein suppression patterns as a converging trait of chronic myeloid leukemia cell adaptation to energy restriction, *Oncotarget.* **7**, 84810-84825.

23. Morin, A., Goncalves, J., Moog, S., Castro-Vega, L. J., Job, S., Buffet, A., Fontenille, M. J., Woszczyk, J., Gimenez-Roqueplo, A. P., Letouzé, E. & Favier, J. (2020) TET-Mediated Hypermethylation Primes SDH-Deficient Cells for HIF2α-Driven Mesenchymal Transition, *Cell reports.* **30**, 4551-4566.e7.

24. Shin, S., Nicolle, R., Jean, C., Samain, R., Ayadi, M., Raffenne, J., Brunel, A., Solorzano, J., Neuzillet, C., Joffre, C., Rocchi, S., Iovanna, J., Dusetti, N., Larsson, O., Pyronnet, S., Bousquet, C. & Martineau, Y. (2021) Translatome-based classification reveals a dual metabolic dependency of a new tumor subtype of pancreatic cancer, *Pancreatology.* **21**, S43.

25. Argüello, R. J., Combes, A. J., Char, R., Gigan, J. P., Baaziz, A. I., Bousiquot, E., Camosseto, V., Samad, B., Tsui, J., Yan, P., Boissonneau, S., Figarella-Branger, D., Gatti, E., Tabouret, E., Krummel, M. F. & Pierre, P. (2020) SCENITH: A Flow Cytometry-Based Method to Functionally Profile Energy Metabolism with Single-Cell Resolution, *Cell metabolism.* **32**, 1063-1075.e7.

26. Giraud, J., Chalopin, D., Blanc, J. F. & Saleh, M. (2021) Hepatocellular Carcinoma Immune Landscape and the Potential of Immunotherapies, *Frontiers in immunology.* **12**, 655697.

27. Kakimoto, P. A., Chausse, B., Caldeira da Silva, C. C., Donato Júnior, J. & Kowaltowski, A. J. (2019) Resilient hepatic mitochondrial function and lack of iNOS dependence in diet-induced insulin resistance, *PloS one.* **14**, e0211733.

28. Hanahan, D. & Weinberg, R. A. (2011) Hallmarks of cancer: the next generation, *Cell.* **144**, 646-74.

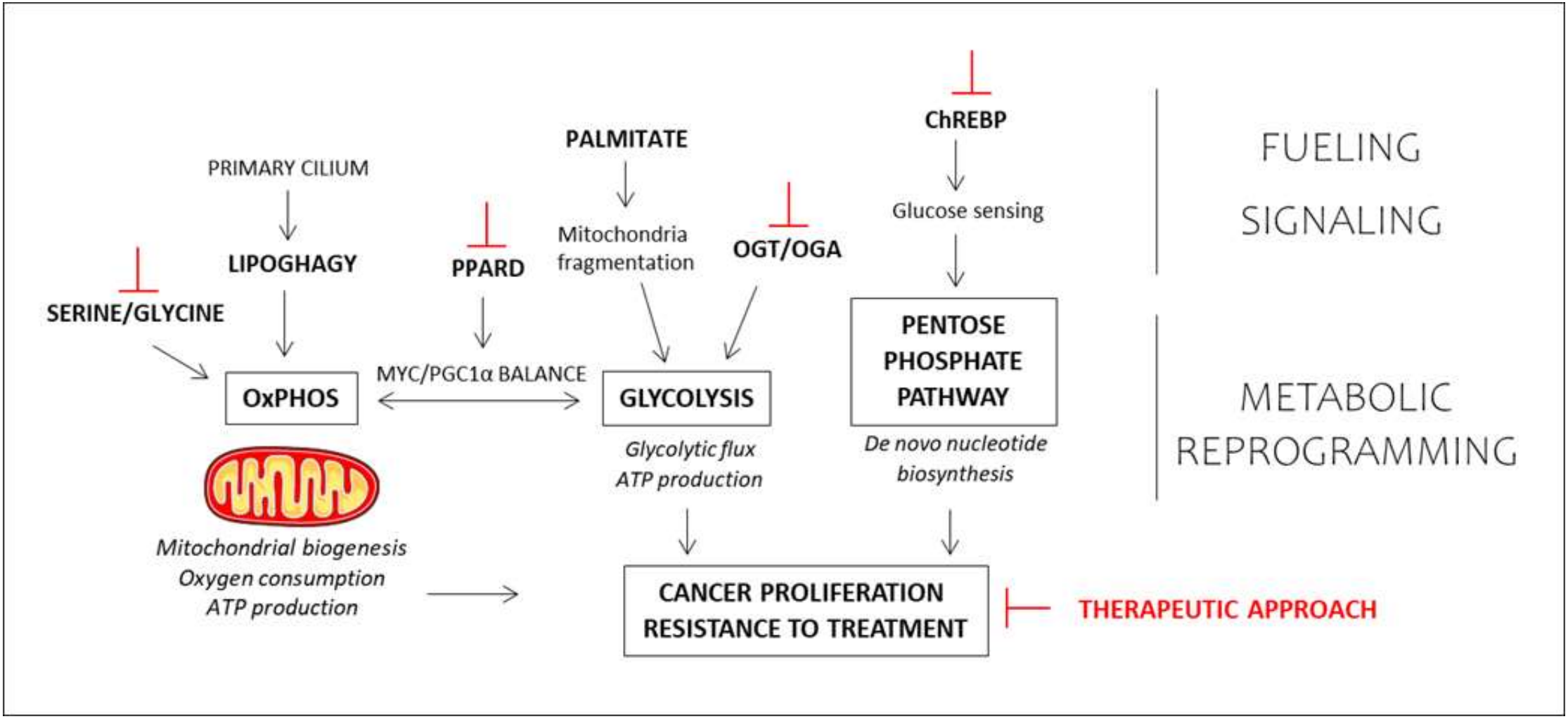
29. Miceli, C., Roccio, F., Penalva-Mousset, L., Burtin, M., Leroy, C., Nemazanyy, I., Kuperwasser, N., Pontoglio, M., Friedlander, G., Morel, E., Terzi, F., Codogno, P. & Dupont, N. (2020) The primary cilium and lipophagy translate mechanical forces to direct metabolic adaptation of kidney epithelial cells, *Nature cell biology.* **22**, 1091-1102.

30. Sancho, P., Burgos-Ramos, E., Tavera, A., Bou Kheir, T., Jagust, P., Schoenhals, M., Barneda, D., Sellers, K., Campos-Olivas, R., Graña, O., Viera, C. R., Yuneva, M., Sainz, B., Jr. & Heeschen, C. (2015) MYC/PGC-1α Balance Determines the Metabolic Phenotype and Plasticity of Pancreatic Cancer Stem Cells, *Cell metabolism.* **22**, 590-605.

31. Tajan, M., Hennequart, M., Cheung, E. C., Zani, F., Hock, A. K., Legrave, N., Maddocks, O. D. K., Ridgway, R. A., Athineos, D., Suárez-Bonnet, A., Ludwig, R. L., Novellasdemunt, L., Angelis, N., Li, V. S. W., Vlachogiannis, G., Valeri, N., Mainolfi, N., Suri, V., Friedman, A., Manfredi, M., Blyth, K., Sansom, O. J. & Vousden, K. H. (2021) Serine synthesis pathway inhibition cooperates with dietary serine and glycine limitation for cancer therapy, *Nature communications.* **12**, 366.

FIGURE LEGEND

Figure 1: Highlights of session 4: Fuel signaling and metabolism. Intermediates: metabolites (serine/glycine, palmitate), cellular processes (Lipophagy), transcription factors (PPARD, CHREB), enzymes (O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA)) driving fuel signaling act on cellular metabolism: OXPHOS, Glycolysis, Pentose Phosphate pathway to regulate proliferation and resistance to treatments.


PRIMARY CILIUM
LIPOGHAGY
SERINE/GLYCINE
PPARD
PALMITATE
Mitochondria fragmentation
OGT/OGA
ChREBP
Glucose sensing
PENTOSE PHOSPHATE PATHWAY
OxPHOS
MYC/PGC1α BALANCE
GLYCOLYSIS
Glycolytic flux
ATP production
De novo nucleotide biosynthesis
Mitochondrial biogenesis
Oxygen consumption
ATP production
CANCER PROLIFERATION
RESISTANCE TO TREATMENT
THERAPEUTIC APPROACH
FUELING SIGNALING
METABOLIC REPROGRAMMING