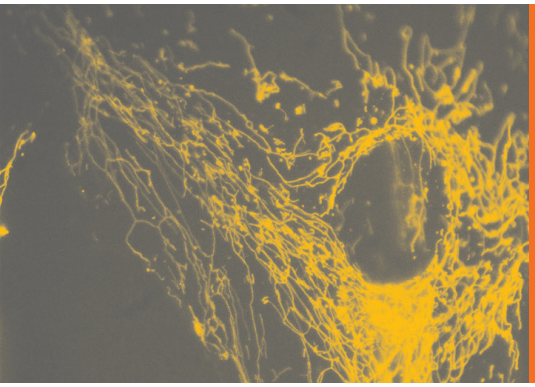
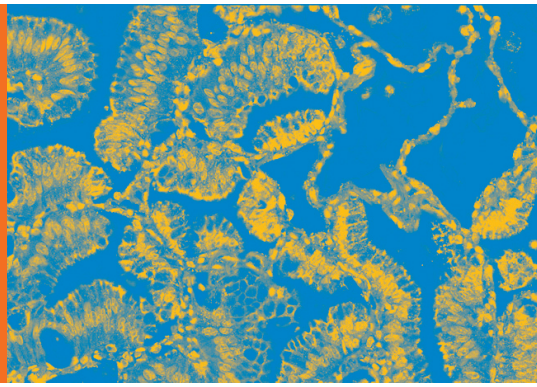
Featuring
Nobel Prize
on Oxygen metabolism,
Sir Peter J. Ratcliffe

VIRTUAL MEETING
May 27th-29th, 2021

4TH EDITION

METABOLISM & CANCER





ABSTRACTS' BOOKLET

DAY 2: FRIDAY, 28TH MAY

METABOLISM-CANCER.COM

BRIO BORDEAUX | Cancéropôle Grand Sud-Ouest | ASPERON & CO LA RECHERCHE SUR LE CANCER ENSEMBLE | NACRe | ONCOSPHERE NOUVELLE-AQUITAINE | université de BORDEAUX Département Sciences Biologiques et Médicales | FONDATION ARC POUR LA RECHERCHE SUR LE CANCER | Région Nouvelle-Aquitaine | RFMF | SFC Société Française du Cancer

Session III
METABOLIC PROFILING AND EPIGENETIC CONTROL
Chairs: Laurent Le Cam (IRCM, Montpellier, France) **& Stéphane Rocchi** (C3M, Nice, France)

Keynote lecture
Novel chromatin based mechanisms

Robert Schneider
Institute of Functional Epigenetics, Helmholtz Zentrum München, Neuherberg, Germany

It has been suggested that post-translational modifications of histones and DNA define distinct chromatin or "epigenetic" states. Nonetheless the set of characterised modifications is far from complete and many modifications on histones, RNA and DNA are awaiting identification and functional characterisation. Additionally, it is currently only poorly understood how mechanistically distinct "epigenetic" (or transcriptional) states are inherited through cellular divisions. We are aiming to i) identify novel players in chromatin function (in particular histone and RNA modifications) and their function and to ii) understand how chromatin states can mediate "epigenetic" memory as well as to iii) how cellular metabolism impacts on chromatin architecture and hence transcription. For this we are applying chromatin biochemistry, different "omics", metabolomics and single cell approaches as well as mathematical modelling in mouse models, mESCs and also yeast cells.

Lecture
Epigenetic reprogramming in SDH-deficient cancers

Judith Favier
Cardiovascular Research Center HEGP, Paris, France

The mitochondrial enzyme succinate dehydrogenase (SDH) is composed of 4 subunits encoded by 4 nuclear genes: *SDHA, SDHB, SDHC and SDHD*. All are tumor suppressor genes predisposing to paraganglioma, pheochromocytoma, GIST, and rare cases of renal cell carcinomas. *SDHB* mutations only are associated with increased risk of metastasis. *SDH l*oss of function is associated with a massive increase in its substrate, succinate, leading to the inhibition of HIF-prolyl hydroxylases and a pseudohypoxic phenotype.
Through the methylome analysis of a large paraganglioma cohort we previously revealed that *SDHx*-related tumors display a hypermethylator phenotype, associated with down-regulation of key genes involved in neuroendocrine differentiation. Epigenetic silencing was shown to be particularly severe in *SDHB*-mutated tumors, potentially explaining their malignancy. We further examined the genome-wide distribution of 5-methylcytosine and 5-hydroxymethylcytosine and their correlation with RNA expression, in *SDHB*-deficient tumors and murine *Sdhb-/-* cells. We demonstrated that DNA hypermethylation results from TET inhibition. Although it preferentially affects PRC2 targets and known developmental genes, PRC2 activity does not contribute to the DNA hypermethylator phenotype. Besides, we evidenced *in vitro* and *in vivo* that TET silencing, while recapitulating the methylation profile of *Sdhb-/-* cells, is not sufficient to drive their EMT-like phenotype, which requires additional HIF2 activation. Further studies, comparing *Sdhb* and *Sdhd*-deficient cells revealed, as in human tumors, an increased hypermethylation following *Sdhb* knock-out, potentially explained by dysregulated iron balance and increased oxidative stress. Altogether, our data suggest that the combination

of succinate and mitochondrial ROS accumulation lead to a strong inhibition of 2-OG-dependent dioxygenases, including TET and PHD enzymes, that act synergistically to promote the acquisition of metastatic traits. These data provide a rationale for targeting HIF2a and DNA methylation in *SDH*-associated malignancies.

Selected talk

Methylglyoxal, a glycolysis by-product, epigenetically silences tumor suppressor genes in triple negative breast cancer

Assia Tiamiou[1], Gaurav Dube[2], Martin Bizet[2], Justine Bellier[1], Yasmine Boumahd[1], Marie-Julie Nokin[1], Rachel Deplus[2], Tom Wissocq[1], Olivier Peulen[1], Vincent Castronovo[1], François Fuks[2], Akeila Bellahcène[1]

1. GIGA Cancer-Metastasis Research Laboratory, University of Liege, Liege, Belgium
2. U-CRC - Laboratory of Cancer Epigenetics, Université Libre de Bruxelles, Brussels, Belgium

INTRODUCTION

Triple-negative breast cancer (TNBC) represents 15%-20% of breast cancers. This subtype has the worst prognosis as it is generally resistant to standard chemotherapy and has no approved targeted therapies. Therefore, it is an urgent need to develop therapies based on novel strategies. TNBC metabolic profiling indicates that this subtype of breast tumors is generally glycolytic. Methylglyoxal (MG) is a very reactive dicarbonyl molecule derived from glycolysis. MG interacts with DNA, lipids and proteins to form Advanced Glycation Products (AGEs). Our previous studies have demonstrated that MG glycating stress triggers enhanced tumor growth and metastasis in breast cancer [1] [2].

MATERIAL AND METHODS

We generated a breast cancer cell line stably depleted for glyoxalase 1 (GLO1), the major enzymatic defense against MG, to induce an endogenous MG stress. We performed RNA sequencing and 850K CpGs array to study gene expression and methylation profiles, respectively. Protein and mRNA expressions of genes of interest was validated using western-blot and RT-QPCR. Moreover, real-time migratory capacity of GLO1-depleted breast cancer cells was evaluated upon 5-Aza (5-aza-2'-deoxycytidine) treatment. Finally, thanks to in silico data we investigate the clinical relevance of MG stress in specific TNBC cohorts.

RESULTS

Transcriptomic analyses revealed a pro-metastatic MG signature comprising the regulation of the expression of several invasion and metastasis-related genes. Among them, the epigenome writers, namely DNA methyltransferase 3A (DNMT3A) and 3B (DNMT3B), were significantly increased suggesting a novel function of MG stress in epigenetic regulation. Consistently, genome wide methylation analysis of GLO1-depleted cells pointed to a significant global hypermethylation. Interestingly, the migratory capacity of GLO1-depleted cells was reversed upon global inhibition of DNMTs with 5-Aza. The integrative analysis of gene expression data with gene enhancer and/or promoter methylation status let us establish an MG stress signature based on the significant loss of several tumor suppressor genes in cancer cells under MG stress. Breast cancer cell-derived MG signature was further evaluated in GSEA cohorts of breast cancer patients. This novel signature allowed the clustering of TNBC patients according to their survival and positively correlated MG score with tumor grade and stemness features in breast cancer.

CONCLUSION

We have demonstrated that MG stress induces the hypermethylation of the promoter/enhancer regions of specific tumor-suppressor genes in TNBC through the overexpression of DNMTs. MG-methylome highlights a novel and unique signature potentially leading to the development of new therapeutic approaches for the treatment of TNBC subtype challenging tumors.

Keywords: methylglyoxal, DNA Methylation, TNBC.

References:
- Nokin *et al. Elife* 2016 Oct 19;5:e19375. doi: 10.7554/eLife.19375
- Nokin *et al. Breast Cancer Res.* 2019 Jan 23;21(1):11. doi: 10.1186/s13058-018-1095-7

Lecture

SCENITH: functional profiling energy metabolism and epigenetics in human samples by Flow cytometry

Rafael Jose Argüello
CIML, Marseille, France

Metabolic interactions between tumor and immune cells represent an emerging hallmark of cancer. However, evidences that support complex metabolic interactions are difficult to obtain because current methods are not suited for studying cells from human and mouse tumors. In order to investigate these questions, we have recently developed and patented a method, called SCENITH that allows to functionally profile metabolism by multi-parametric flow cytometry.Our method allows for metabolic deconvolution and paralleled metabolism and global epigenetic analysis of all cells present in a sample. SCENITH is performed rapidly *ex vivo* and allows to determine in parallel the cellular composition, the phenotype and the metabolic profile multiple cell types present in one sample. We used this method to analyze human blood, lymph nodes and human tumors paralleled with single cell RNA-seq and SCENITH of the same human tumor samples. We identified a small set of metabolic genes that strongly correlate with functional metabolic profile of human myeloid cells. Moreover, we identified a conserved metabolic and epigenetic evolution of monocytes to macrophages differentiation upon migration into tumors. SCENITH™ ability to rapidly determine complex and linked metabolic activities in discrete cell subpopulations within tissue or blood draws will contribute to the functional information needed for evaluating therapeutic responses or patient stratification.

Altogether, SCENITH represent a functional method for paralleled phenotypic and metabolic immunoprofiling with great potential to be incorporated in the future to the personalized medicine toolbox.

Industry talk

NextGen-O2k and bioenergetics communications

Erich Gnaiger
Oroboros Instruments innovations

Selected talk

Translatome-based classification reveals a dual metabolic dependency of a new tumor subtype of pancreatic cancer

Yvan Martineau[1], Sauyeun Shin[1], Remy Nicolle[2], Christine Jean[1], Rémi Samain[1], Mira Ayadi[2], Jérome Raffenne[1], Alexia Brunel[1], Jacobo Solorzano[1], Cindy Neuzillet[3], Carine Joffre[1], Stephane Rocchi[4], Juan Iovanna[5], Nelson Dusetti[5], Ola Larsson6[5], Stéphane Pyronnet[1], Corinne Bousquet[1]

1. CRCT, Toulouse, France
2. Ligue Nationale Contre Le Cancer, Paris, France
3. Institut Curie, Saint-Cloud, France
4. C3M, Nice, France
5. CRCM, Marseille, France
6. Karolinska Institute, Stockholm, Sweden

INTRODUCTION

Molecular profiling of Pancreatic Ductal Adenocarcinoma (PDA), based on transcriptomic analyses, identifies two main prognostic subtypes (basal-like and classical), but does not allow personalized first-line treatment. To date, tumors have not been profiled based on protein synthesis rates, yet the step of mRNA translation is highly dysregulated in both PDA cancer cells and their microenvironment.

AIM

We aim at assessing whether quantification of mRNA translation could provide a distinct perspective on PDA and identify novel tumor subtypes.

MATERIALS AND METHODS

Using a collection of twenty-seven pancreatic Patient-Derived Xenografts (PDX), we performed transcriptome-wide analysis of translated mRNA (translatome). Unsupervised bioinformatics analysis allowed PDA tumors classification according to mRNA translation rate. PDX-derived cancer cells as well as common PDA cell lines were used to functionally characterize newly identified subtype.

RESULTS

Independent component analysis revealed a new tumor subtype harboring a low protein synthesis rate, but associated with a robust translation of mRNAs encoding effectors of the integrated stress response (ISR), including the transcription factor ATF4. Functional characterization of the "ISR-activated" human cancer cells revealed a high resistance to drugs, low autophagic capacities, and importantly, metabolic impairments in the serine synthesis and transsulfuration pathways. Therefore, the drug-resistant cancer cell phenotype showing auxotrophy to both serine and cysteine may be amenable to targeted therapy.

CONCLUSION

Overall, our study highlights profiling of mRNA translation in cancer as an underexplored avenue for identification of previously unrecognized subtypes together with potential treatments.

Keywords: pancreatic cancer, mRNA translation, PDX, integrated stress response, serine metabolism.

References:
- Müller D. Larsson O., Bousquet C., Pyronnet S., Martineau Y. *eIF4A inhibition circumvents uncontrolled DNA replication mediated.* PMID 31672935.
- Duluc C. Pyronnet S., Martineau Y., Bousquet C. *Pharmacological targeting of the protein synthesis mTOR/4E-BP1 pathway in cancer-associated fibroblasts abrogates pancreatic tumour chemoresistance. EMBO Mol Med* 2015. PMID 25834145

Selected talk

Interaction of LncRNA LENOX with the GTPase RAP2C promotes oxidative phosphorylation, melanoma cell survival and resistance to BRAF inhibition

Giovanni Gambi[1], Guillaume Davidson[1], Alessandro Cuomo[2], Tiziana Bonaldi[2], Vicky Katopodi[3], Eleonora Leucci[3], Irwin Davidson[1]

1. Functional genomics and cancer, IGBMC, Illkirch, France
2. IEO, Milan, Italy
3. KU Leuven, Leuven, Belgium

INTRODUCTION

Recent years have witnessed the increasing recognition of the essential roles of long intergenic non-coding (Linc)RNAs in many biological processes including oncogenic transformation and tumor progression. Malignant melanoma is the most aggressive skin cancer and despite the development of target- and immunotherapies many patients do no respond to available treatments. LincRNA targeting by antisense oligonucleotides (ASOs) represents a potential new therapeutic strategy[1]. The lineage-defining transcription factors MITF (Microphthalmia-associated transcription factor) and SOX10 promote melanoma cell proliferation and survival by regulating a repertoire of protein coding genes and the SAMMSON lincRNA[2]. We identified LENOX (LincRNA-ENhancer of OXidative phosphorylation) as an additional MITF/SOX10 target essential for melanoma cell viability and provided a model for its mechanism of action.

METHODS

We evaluated LENOX expression over different public bulk and single cells RNA sequencing datasets. We confirmed these analyses with independent methods (fluorescence in situ hybridization/FISH, quantitative PCR/qPCR). We monitored the effects of LENOX loss of function by ASOs/CRISPR interference or shRNAs and gain of function by controlled overexpression using different cellular assays (cell counting, crystal violet staining, flow cytometry, immunofluorescence/IF). We performed LENOX isolation using biotinylated probes and identified its protein partners by mass spectrometry. Interaction with RAP2 was confirmed by RNA immunoprecipitation. LENOX and RAP2 localization were evaluated by FISH coupled with IF and western blot/qPCR analyses of mitochondria isolated by fractionation. Mitochondrial function in LENOX knock down or -overexpressing cells was monitored by measurement of oxygen consumption rate (using the Seahorse instrument), flow cytometry and IF (mitotracker, CellROX stainings). DRP1 phosphorylation status was evaluated by western blot.

RESULTS

LENOX was expressed *in vivo* by normal skin melanocytes and significantly upregulated in melanoma, irrespective of mutational status and phenotype (melanocytic/undifferentiated). LENOX was also frequently amplified and associated with poor prognosis features. Its knock down by ASOs reduced melanoma cells proliferation and induced apoptosis, with a synergistic effect when SAMMSON was co-targeted. LENOX overexpression increased melanoma cells growth and clonogenic capacity. We further showed that LENOX

promoted association of the RAP2C GTPase with DRP1 enhancing DRP1 S637 phosphorylation, mitochondrial fusion and oxidative phosphorylation while limiting ROS production. LENOX was also transcriptionally up-regulated upon treatment with MAP kinase inhibitors and cooperated with RAP2C to promote the ensuing metabolic switch from glycolysis to oxidative phosphorylation. Consequently, LENOX and RAP2C silencing cooperated with MAP kinase inhibitors to induce melanoma cell apoptosis.

CONCLUSIONS

We showed that LENOX and SAMMSON act cooperatively to support melanoma cell survival. By mediating RAP2C/DRP1 interaction, LENOX balances ERK1/2 dependent mitochondrial fragmentation[3], thus supporting mitochondrial health in basal condition. Upon metabolic stress (such as after MAPK inhibition[4]) LENOX induction is important to sustain mitochondrial fusion and increase oxidative phosphorylation as a savage strategy to glycolysis inhibition. We thus characterized a novel lincRNA-protein complex important for melanoma cells metabolism. LENOX targeting by ASOs could constitute a novel therapeutic strategy, as a single agent or in combination with SAMMSON specific ASOs or MAPK inhibitors.

Keywords: metabolism, mitochondrial homeostasis, drug resistance, GTPase, signalling, melanoma.

References:

1. Anaïs M. Quemener *et al. The powerful world of antisense oligonucleotides: From bench to bedside. Wiley Interdisciplinary Reviews*: RNA (2020).
2. Eleonora Leucci et al. Melanoma addiction to the long non-coding RNA SAMMSON. Nature (2016).
3. Madhavika N. Serasinghe *et al. Mitochondrial division is requisite to RAS-induced transformation and targeted by oncogenic MAPK pathway inhibitors. Molecular Cell* (2015).
4. Madhavika N. Serasinghe *et al. Dual suppression of inner and outer mitochondrial membrane functions augments apoptotic responses to oncogenic MAPK inhibition. Cell Death and Disease* (2018).

Short presentation of Société Française du Cancer (SFC)

Julie Pannequin
(SFC)

Session IV
SIGNALING, FUELING & METABOLISM IN CANCER

Chairs: Frédéric Bost (C3M, Nice, France) **& Jean-Emmanuel Sarry** (CRCT, Toulouse, France)

Lecture
Metabolic control of immunity by the hexosamine biosynthetic pathway and O-GlcNacylation

Maya Saleh
Oncoimmunology Research, ImmunoConcEpT Laboratories,
Department of Life Sciences and Health, The University of Bordeaux, France

Cellular metabolism has emerged as a crucial determinant of inflammation and immunity. Like cancer, inflammation is energetically expensive and imposes a significant metabolic stress on the body. It is thus

hypothesized that metabolic alterations contribute to inflammatory disease pathogenesis, tumor immune escape and morbidities. Myeloid cells play a central role in inflammation and innate immunity. Their "classical" activation, which is associated with enhanced phagocytosis, microbicidal activity and production of pro-inflammatory cytokines and reactive oxygen species (ROS) is accompanied by a reliance on aerobic glycolysis, due to nitric oxide (NO)-mediated mitochondrial collapse, and the accumulation of tricarboxylic acid (TCA) cycle intermediates such as succinate, citrate, fumarate and itaconate, which are emerging as important regulators of inflammation and innate immunity. In contrast, "alternative" polarization of macrophages into an "anti-inflammatory" state promotes their tissue repair functions and pro-tumorigenic activities. Such macrophage commitment appears to rely on enhanced glutamine catabolism and flux through the hexosamine biosynthetic pathway (HBP), as determined by transcriptional and metabolic profiling. The HBP is a branch of glycolysis that consumes 2-5% of cellular glucose. It also integrates signals from glutamine, fatty acid (acetyl-coA) and nucleotide (UTP) metabolism. Through production of the high-energy metabolite uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), HBP links nutrient sensing into a tractable cellular modification of substrate proteins, namely O-GlcNAcylation. This dynamic post-translational modification of serine/threonine (S/T) residues in target proteins often overlaps with phosphorylation. However, unlike the latter, which is orchestrated by >600 S/T kinases and phosphatases, O-GlcNAcylation is regulated by two unique cellular enzymes, O-GlcNAc transferase (OGT), which catalyzes the addition of O-GlcNAc to substrates and O-GlcNAcase (OGA) that hydrolyzes it. The tight regulation of O-GlcNAc cycling and its physiological importance are best illustrated by the cross-regulation of OGT and OGA and the lethal phenotypes of *Ogt -/-* and *Oga -/-* mice. Several recent lines of evidence link augmented HBP flux and O-GlcNAcylation to inflammation and immunity: a) O-GlcNAcylation regulates pathways associated with the Warburg effect and inflammation, through stimulatory modification of glycolysis enzymes, and transcription factors e.g. HIF-1α and c-Myc; b) It is upregulated in activated T cells, where it is required for self-renewal, malignancy, peripheral clonal expansion and IL-2 production; c) It is similarly required for B cell homeostasis and antibody production; and d) It regulates central effectors of inflammatory and metabolic signaling pathways, notably it inhibits Ripk3 and S6k phosphorylation in macrophages, blunting their classical activation. In my talk, I'll discuss our recent results on the role of HBP in systemic lupus erythematosus (SLE). I'll also present results obtained using myeloid-specific knockout of *Ogt* in a model of glioma. Collectively, our work point to the HBP as a potential therapeutic target to modulate inflammation and anti-tumor immunity.

Lecture

Dietary fatty acids and the regulation of glycolysis

Alicia Kowaltowski

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Brazil

Palmitic acid is the most abundant saturated fatty acid in human serum. In cell culture systems, palmitate overload is considered a toxic stimulus, and promotes lipid accumulation, insulin resistance, endoplasmic reticulum stress, oxidative stress, as well as cell death. An increased supply of fatty acids has also been shown to change the predominant form of the mitochondrial network, although the metabolic effects of this change are still unclear. We aimed to uncover the early bioenergetic outcomes of lipotoxicity. We incubated hepatic cells with palmitate conjugated to BSA and followed real-time oxygen consumption and extracellular

acidification for 6 hours. Palmitate increased glycolysis as soon as 1 hour after the stimulus, while oxygen consumption was not disturbed, despite overt mitochondrial fragmentation and cellular reductive imbalance. Palmitate only induced mitochondrial fragmentation if glucose and glutamine were available, while glycolytic enhancement did not require glutamine, showing it is not dependent on morphological changes. NAD(P)H levels were significantly abrogated in palmitate-treated cells. Knockdown of the mitochondrial NAD(P) transhydrogenase or addition of the mitochondrial oxidant-generator menadione in control cells modulated ATP production from glycolysis. Indeed, using selective inhibitors, we found that the production of superoxide/hydrogen peroxide at the IQ site of electron transport chain complex I is associated with the metabolic rewiring promoted by palmitate, while not changing mitochondrial oxygen consumption. In conclusion, we demonstrate that increased glycolytic flux linked to mitochondrially-generated redox imbalance is an early bioenergetic result of palmitate overload and lipotoxicity.

Selected talk

Role of the transcription factor to liver carcinogenesis

Emmanuel Benichou, Bolaji Seffou Bolaji Seffou, Selin Topcu, Véronique Lenoir, Carina Prip-Buus, Marie-Clotilde Alves-Guerra, Sandra Guilmeau, Renaud Dentin
Institut Cochin, INSERMU1016, CNRS UMR8104, Universié de Paris, Paris, France

INTRODUCTION

Primary liver cancer is a global public health concern as it represents one of the most lethal human malignancies observed worldwide. While surgical resection and liver transplantation are effective options in the treatment of early-stage disease, therapeutic approaches for advanced HCC are very limited. Thus, the elucidation of the molecular pathogenesis of HCC is imperative for the development of alternative therapeutic strategies with improved potency.

Because specific altered metabolic features are altered across many cancer types, reprogrammed metabolism is considered a hallmark of cancer. Specifically, changes in tumor bioenergetics, consisting of elevation of glycolysis, upregulation of lipid and amino acid metabolism and induction of the pentose phosphate pathway, are detected in most cancer types and often negatively correlated with survival prognosis. Overall, the key question driving research in the field is to identify key metabolic candidates whose inactivation will impair hepatocarcinogenesis while sparing normal cells for therapeutic benefits. In this context, we have previously established that the glucose responsive transcription factor ChREBP (Carbohydrate Responsive Element Binding Protein) plays a central role in the regulation of multiple metabolic pathways in nonproliferating hepatocytes. ChREBP is a major mediator of glucose action on glycolytic, pentose phosphate and lipogenic gene expression. Therefore, given its key role in the control of energy metabolism, ChREBP represents a promising candidate for targeted therapies during HCC treatment.

METHODS

This study was designed to determine the contribution of ChREBP to HCC initiation and development. First, its contribution to liver carcinogenesis was assessed by using 10 publicly available HCC human datasets. In addition, we characterized its oncogenic function at the molecular level by taking advantage of the sleeping beauty transposon system to stably overexpress ChREBP in the liver of C57bl6/J mice.

RESULTS

Our study demonstrates that enhanced ChREBP activity is sufficient to initiate the development and progression of HCC, unraveling its oncogenic function in the liver. In agreement, ChREBP expression signs HCC tumors with poor prognosis in human. At the molecular level, our study unravels that ChREBP activation enhances in a p85a-dependent manner the PI3K/AKT signaling, which contributes to its pro-proliferative effect. ChREBP also coordinately rewires both glucose and glutamine metabolic fluxes to enhance *de novo* nucleotide and fatty acid synthesis to sustain cell proliferation. Therefore, these findings delineate an undescribed ChREBP-regulating circuitry through which HCC cells ensure balanced coordination between PI3K/AKT signal transduction and appropriate cell anabolism to initiate and support HCC development. Finally, the fact that ChREBP activation is an important feature of hepatic cancer cell proliferation and survival, provides a unique opportunity for the development of new therapeutics for HCC treatment. In this context, we identified and characterized the first ChREBP inhibitor, that prevents the activation of oncogenic signaling pathways and compensatory cell metabolic rewiring. As a consequence, upon ChREBP pharmacological inhibition, tumor development was decreased, and HCC cells were also sensitized to chemotherapy treatment.

CONCLUSION

Overall, ChREBP represents a strong candidate for pharmacological intervention since targeting its activity serves as an anti-neoplastic strategy and as a promising approach to counteract chemotherapy resistance during HCC treatment.

Keywords: ChREBP, HCC, metabolic rewiring, glucose, glutamine, nucleotides.

Industry talk

Analysis of rates of cellul ar bioenergetics pathways and applications in studying metabolic reprogramming of cancer cells

Barry Bochner
Biolog Inc

Lecture

Autophagic and primary cilium machineries crosstalk in mechanical stress sensing: molecular and functional aspects

Etienne Morel
Institut Necker Enfants Malades, Paris, France

Autophagy is a conserved catabolic pathway directly involved in the degradation and recycling of intracellular components. The autophagic process is associated with a response to stress situations such as chemical toxicity, pathogens entry, nutrients deficit and mechanical stresses, including shear stress induced by biological fluids. In kidney epithelial cells, the constant primitive urine flow induces shear stress with important consequences on cellular adaptation and differentiation, such as cell volume regulation. We previously reported that the sensing of shear stress in kidney cells is orchestrated by a specific dialog between the sensory organelle primary cilium and the autophagic machinery. Here we show that shear

stress favors mitochondrial biogenesis and metabolic reprogramming to ensure proper energy production and cellular adaptation to constant liquid flow. We show that shear stress, via a primary cilium dependent process, induces lipophagy, a specialized autophagic program, thus contributing to fatty acids production that provides mitochondrial substrates to generate ATP through beta-oxidation. Altogether, our results demonstrate that the primary cilium – autophagy dialog in response to shear stress enables the proper translation of mechanical forces into metabolic adaptation.

Selected talk

PPARD integrates microenvironmental signals to activate a pro-metastatic metabolic program in pancreatic cancer

Beatriz Parejo-Alonso[1], David Barneda[2], Sara Trabulo[2], Sara Compte-Sancerni[2], Pilar Espiau-Romera[1], Alba Royo-García[1], Sarah Courtois[1], Christopher Heeschen[3], Patricia Sancho[1]

1. Immunity, Cancer and infectious origin or molecular base diseases, IIS Aragón, Zaragoza, Spain
2. Centre for Stem Cells in Cancer & Ageing, Barts Cancer Institute, London, United Kingdom
3. Centre for Single-Cell Omics and Key Laboratory of Oncogenes and Related Genes, Shanghai, China

INTRODUCTION

Previous data from our group suggest that MYC/PGC1A balance modulates the metabolic phenotype in pancreatic cancer, directly controlling stemness and invasiveness in response to energy deprivation and environmental cues. Since no specific MYC inhibitors are currently available, we decided to focus on directly targetable upstream signals regulating the metastatic process.

MATERIALS AND METHODS

Cells: primary cultures from PDAC-derived xenografts (PDXs).

EMT induction: treatment with a panel of EMT inducers (UK5099, Malonate, Etomoxir, tumor-like conditions or M2- polarized conditioned medium (MCM)); pharmacologic activation of PPARA (Rosiglitazone), PPARD (L-165, GW0742, GW501516) and PPARG (WY14643); pharmacologic inhibition of PPARD (GSK0660, GSK3787, DG172). Gene expression profile: quantitative PCR (qPCR) for epithelial-to-mesenchymal transition (EMT)-related genes assessment and MYC/PGC1A ratio determination.

Pro-metastatic abilities: invasion assays in Boyden Chambers; experimental metastasis assay *in vivo* after injections in the spleen or the pancreas. Genetic modulation: PPARD and MYC overexpression and/or knockdown. MYC and PGC1A regulation by PPARD: promoter activity assessment.

Real time metabolic analysis: glycolytic capacity and mitochondrial respiration through Seahorse® Extracellular Flux Analyzer.

Statistical analysis: Mann-Whitney test or Student's t-test were used for 2 groups comparisons and Kruskal-Wallis test or one-way analysis of variance (ANOVA) for multiple comparisons. Data were considered significant when p-value < 0.05.

RESULTS

Peroxisome Proliferator-activated Receptor Delta (PPARD) was consistently upregulated in response to a panel of EMT inducers (i.e. macrophage-derived conditioned medium or low dose Etomoxir) prior to EMT-related transcriptional changes. Intriguingly, treatment with various PPARD agonists, but not PPARA

o PPARG agonists, induced the expression of EMT-related genes and increased MYC/PGC1A ratio. These expression changes were translated into morphological changes, and enhanced invasiveness *in vitro* and metastasis *in vivo*. Mechanistically, PPARD overexpression or incubation with the agonist GW0742 induced an early activation of MYC promoter, reducing the activity of PGC1A promoter. Moreover, MYC knockdown blocked invasion induced by PPARD, suggesting its pro-metastatic effects were dependent on MYC/PGC1A. Conversely, PPARD knockdown prevented EMT-induced expression changes, metabolic switch and enhanced invasiveness. Consistently, pharmacological PPARD targeting inhibited the invasive capacity conferred by EMT inducers previously described or basal invasiveness in highly metastatic cells. Importantly, *in vivo* treatment with the PPARD antagonist GSK3787 showed decreased liver metastases incidence, which strongly supports the pro-metastatic role of PPARD in PDAC and opens future therapeutic perspectives for the treatment of this deadly disease.

CONCLUSIONS

PPARD integrates microenvironmental signals to reprogram cellular metabolism via MYC/PGC1A to promote cancer cell invasiveness and metastasis.

Keywords: EMT, MYC, PPARD, PGC1a, metabolic reprogramming, PDAC.

Keynote lect ure

Dietary modulation and cancer therapy

Karen Vousden, Mylene Tajan, Marc Hennequart
The Francis Crick Institute, London, United Kingdom

There is compelling evidence that diet can affect cancer development, and an increasing interest in the concept that dietary manipulation could contribute to cancer therapy. We have found that many cancer cells depend on an exogenous supply of serine for optimal growth. Dietary serine and glycine depletion can lower circulating serine levels and reduce the growth of some tumours. However, alterations in cancer cells that lead to the activation of the *de novo* serine synthesis pathway result in resistance to dietary serine and glycine depletion. We have tested the ability of a small molecule inhibitor of PHGDH, the first step in the serine synthesis pathway, to improve the response to dietary intervention. We are also exploring how regulation of other circulating metabolites, such as formate, regulate cancer development and progression.