ISCaM 2019
6th Annual Meeting
17-19 October
Braga, Portugal

CANCER METABOLIC REWIRING: MAPPING THE ROAD TO CLINICAL TRANSLATION

international society of cancer metabolism

www.iscams.org

SESSION TOPICS

- **Cancer pH Dynamics**
- **Genetic and Epigenetic Regulation of Cancer Metabolism**
- **Metabolism and Cancer Heterogeneity**
- **Metabolism and Cancer Progression**
- **Metabolism and Regulated Cell Death**
- **Diet, Microbiota and Cancer Metabolism**
- **Cancer Metabolism and Immune Response**
- **Target Metabolism in Cancer Therapy**

Keynote Speakers

Jacques Pouysségur
Centre Scientifique de Monaco, MC

Diane Barber
University of S. Francisco, USA

Invited Speakers

Ana Gil
University of Aveiro, PT

Christian Frezza
University of Cambridge, UK

Aung Naing
University of Texas, USA

Ciro Isidoro
University of Piemonte Orientale, IT

Cristina Muñoz-Pinedo
Bellvitge Biomedical Research Institute
L'Hospitalet, ES

Fatima Mechta-Grigoriou
INSERM, FR

Fátima Martel
University of Porto, PT

Jacinta Serpa
New University of Lisbon, PT

Javier A Menendez
Catalan Institute of Oncology, ES

Marja Jäättelä
University of Copenhagen, DK

Marina Kreutz
University Hospital Regensburg, DE

Massimiliano Mazzone
VIB - KU Leuven, BE

Pawel Swietach
University of Oxford, UK

Sarah Halford
Cancer Research, UK

Valerio Pazienza
IRCCS - Hospital San Giovanni Rotondo, IT

Tea Pemovska
CeMM - Research Center for Molecular Medicine of the Austrian Academy of Sciences, AT

WELCOME MESSAGE

Dear friends and colleagues,

On behalf of the organizing committee, it is our pleasure to welcome you in Braga, Portugal, for the "6th Annual ISCaM Meeting 2019". In this meeting, we have set a comprehensive scientific program that tackles the many faces of cancer metabolism, highlighting the recent advances in the field and the road ahead for clinical translation.
The meeting venue is at Bom Jesus do Monte Sanctuary complex, one of the most iconic places of Portugal, that this year was recognized a UNESCO World Heritage cultural site.
The social events include a welcome cocktail with the sound of Minho folk music, a visit to Porto where we will have a guided tour to a wine cellar, followed by Port wine tasting and dinner at the sea side.
We hope that this meeting will offer the opportunity to exchange ideas in a friendly and stimulating environment, promoting fruitful collaborations.
We wish you a very pleasant stay in our beautiful country and expect this to be an inspirational and memorable ISCaM meeting!

Your local hosts,

Ana Preto

Universidade do Minho
Escola de Ciências

cbma centre of molecular and environmental biology

Fátima Baltazar

Universidade do Minho
Escola de Medicina

ICVS

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Program

01

08:30 » 10:00 Registration

10:00 » 11:00 **Welcome**
Fátima Baltazar ICVS - University of Minho; **Ana Preto** CBMA - University of Minho; **Vítor Veloso** President of LPCC - Núcleo Regional do Norte; **Carla Martins** Pro-Rector, University of Minho; **Ricardo Rio** Braga City Mayor

11:00 » 12:00 **Opening lecture** *LPCC-NRN sponsored lecture*
Diane Barber University of S. Francisco, USA
Intracellular pH Regulation of Protein Dynamics: From Cancer to Stem Cell Behaviors

12:00 » 14:00 **Lunch, poster viewing** *Sala Colunata*
& Meet the Professor Diane Barber *13:30 » Sala Templo, Hotel do Templo*

14:00 » 15:30 **Session 1: Cancer pH Dynamics**
Chairs: **Pierre Sonveaux** Université Catholique de Louvain, UCLouvain, BE;
Sofia Avnet University of Bologna, IT

Invited lectures

14:00 » 14:30 **Pawel Swietach** University of Oxford, UK
Exploring acid handling and sensing mechanisms in a large panel of colorectal cancer cells

14:30 » 15:00 **Marja Jäättelä** University of Copenhagen, DK
Lysosomal control of cytosolic pH

Selected communications

15:00 » 15:15 **Surviving metabolism: acidity as a selection pressure in colorectal cancer cell lines**
Johanna Michl University of Oxford, UK

15:15 » 15:30 **Acid adaption of cancer cells rewires pH homeostasis, lipid metabolism and lysosomal biogenesis**
Stine Falsig Pedersen University of Copenhagen, DK

15:30 » 17:00 **Session 2: Genetic and Epigenetic Regulation of Cancer Metabolism**
Chairs: **Carmen Jerónimo** IPO-Porto, PT; **Joana Paredes** I3S, PT

Invited lectures

15:30 » 16:00 **Christian Frezza** University of Cambridge, UK *LPCC-NRN sponsored lecture*
Mitochondrial metabolites and cancer

16:00 » 16:30 **Javier A Menendez** Catalan Institute of Oncology, ES
Metformin: a metabolo-epigenetic drug for cancer therapy

Selected communications

16:30 » 16:45 **Acetyl-CoA metabolism supports multi-step pancreatic carcinogenesis**
Alessandro Carrer Venetian Institute of Molecular Medicine, IT

16:45 » 17:00 **Regulation of energy metabolism by formate**
Alexei Vazquez Beatson Institute, UK

17:00 » 17:30 Coffee Break

17:30 » 19:00 **Session 3: Metabolism and Cancer Heterogeneity**
Chairs: **Stine Falsig Pedersen** University of Copenhagen, DK;
Paulo Oliveira University of Coimbra, PT

Invited lectures

17:30 » 18:00 **Fatima Mechta-Grigoriou** INSERM, FR
Role of oxidative stress and stromal heterogeneity in cancer

18:00 » 18:30 **Jacinta Serpa** New University of Lisbon, PT
Cancer cells metabolic heterogeneity underlies chemoresistance

Selected communications

18:30 » 18:45 **PI3K-C2Y Loss Promotes Pancreatic Cancer through mTOR Regulation and Metabolic Rewiring**
Maria Chiara De Santis University of Torino, Molecular Biotechnology Center, IT

18:45 » 19:00 **Metabolic intratumoural heterogeneity in cancer**
Maria Colman UMC Utrecht, NL

19:00 » 21:00 Welcome Reception with Minho folk music

20:30 ISCaM Board meeting *Sala Templo, Hotel do Templo*

08:00 » 09:30 — **Session 4: Metabolism and Cancer Progression**
Chairs: **Bruno Costa** ICVS, PT; **Lucie Brisson** INSERM, University of Tours, FR

Invited lectures

08:00 » 08:30 — **Ana Gil** University of Aveiro, PT
Metabolomics and the unveiling of new metabolic players in cancer

08:30 » 09:00 — **Ciro Isidoro** University of Piemonte Orientale, IT
Glucose metabolism and autophagy in cancer progression

Selected communications

09:00 » 09:15 — **Characterization of the metabolic control of brain metastasis in breast cancer**
Marine CNM Blackman UCLouvain, BE

09:15 » 09:30 — **Loss of ISG15 expression and ISGylation Reduces Mitophagy and the Functionality and Metabolic Plasticity of Pancreatic Cancer Stem Cells**
Bruno Sainz Instituto de Investigaciones Biomédicas, ES

09:30 » 10:00 — **Coffee Break**

10:00 » 11:30 — **Session 5: Metabolism and Regulated Cell Death**
Chairs: **Paula Ludovico** ICVS, PT; **Valdemar Máximo** I3S, PT

Invited lectures

10:00 » 10:30 — **Cristina Muñoz-Pinedo** Bellvitge Biomedical Research Institute L'Hospitalet, ES
A chemotactic and pro-inflammatory signature induced by starvation and anti-metabolic therapy

10:30 » 11:00 — **Tea Pemovska** CeMM - Research Center for Molecular Medicine of the Austrian Academy of Sciences, AT
Identification and targeting of metabolic vulnerabilities in myeloid leukemias

Selected communications

11:00 » 11:15 — **P53 sustains oxidative metabolism and delays the energetic collapse caused by targeting respiratory complex I in combination with low glucose**
Giuseppe Gasparre University of Bologna, IT

11:15 » 11:30 — **Role of autophagy in the interactions between adipocytes and breast cancer cells**
Lucie Brisson INSERM, University of Tours, FR

11:30 » 12:00 — **Meet the Professor Jacques Pouysségur** *Sala Templo, Hotel do Templo*
& poster viewing *Sala Colunata*

12:00 » 13:30 — **Lunch & poster viewing** *Sala Colunata*

13:30 » 14:30 — **ISCaM General assembly**

14:30 » 16:00 — **Session 6: Diet, Microbiota and Cancer Metabolism**
Chairs: **Margarida Casal** CBMA, PT; **Egídio Torrado** ICVS, PT

Invited lectures

14:30 » 15:00 — **Valerio Pazienza** IRCCS - Hospital San Giovanni Rotondo, IT
Pharmacomicrobiomics: exploiting the diet-drug-microbiota interactions in anticancer therapies

15:00 » 15:30 — **Fátima Martel** University of Porto, PT
Effect of dietary polyphenols on nutrient uptake: a new potential therapeutic target for cancer

Selected communications

15:30 » 15:45 — **Obesity and Triple Negative Breast Cancer: is apelin a new key target?**
Florian Gourgue UCLouvain, BE

15:45 » 16:00 — **Polyunsaturated fatty acids reduce in vitro tumor growth of colorectal cancer patient-derived organoids**
Marianela Vara-Messler University of Turin, IT

16:00 » 16:30 — **The interaction between the vaginal microbiome, HPV infection and cervical cancer**
Pedro Vieira Baptista ISSVD, Unilabs; **Carlos Sousa** LAP-Unilabs — *Sponsor lecture UNILABS*

16:30 » 17:00 — **Coffee Break**

16:45 — **Departure to Porto**

18:30 — **Visit to Taylor´s Porto wine Cellars**

20:30 — **Conference Dinner** *Praia da Luz, Foz - Porto*

09:00 » 10:30 — **Session 7: Targeting Metabolism in Cancer Therapy**
Chairs: **Lúcio Santos** IPO-Porto, PT; **Nicola Baldini** University of Bologna, IT

Invited lectures

09:00 » 09:30 — **Aung Naing** University of Texas, USA — *ASPIC sponsored lecture*
Targeting metabolism and immune response from tissue to liquid biopsy

09:30 » 10:00 — **Sarah Halford** Cancer Research, UK — *ASPIC sponsored lecture*
Remove The Road Blocks: A multi-disciplinary approach for the development of the first-in-human, first-in-class monocarboxylate transporter 1 (MCT1) inhibitor, AZD3965, targeting patients with advanced solid tumours and DLBCL

Selected communications

10:00 » 10:15 — **Epigenetic-metabolic interplay in renal cell carcinoma: role of lactate on sirtuin's modulation**
Vera Miranda-Gonçalves IPO-Porto, PT

10:15 » 10:30 — **Genetic disruption of the cystine importer xct (SLC7A11) reduces growth, survival and tumorigenicity and increases sensitivity to chemotherapy of PDAC cells (CAPAN-2 and MIAPACA-2)**
Daher Boutaina Scientific Centre of Monaco, MC

10:30 » 11:00 — **Coffee Break**

11:00 » 12:30 — **Session 8: Cancer Metabolism and Immune Response**
Chairs: **Ricardo Silvestre** ICVS, PT; **Maria Oliveira** I3S, PT

Invited lectures

11:00 » 11:30 — **Marina Kreutz** University Hospital Regensburg, DE
Metabolic checkpoints in the tumor environment

11:30 » 12:00 — **Massimiliano Mazzone** VIB - KU Leuven, BE — *LPCC-NRN sponsored lecture*
Harnessing tumor metabolism to overcome immunosuppression

Selected communications

12:00 » 12:15 — **Inhibition of glutamine synthetase by glufosinate ammonium skews macrophages towards a M1-like phenotype and inhibits tumor metastasis**
Alessandra Castegna University of Bari, IT

12:15 » 12:30 — **New target molecules depict T regulatory cells in Malignant Mesothelioma**
Stefania Oliveto National Institute of Molecular Genetics, IT

SATURDAY, 19TH OCTOBER

12:30 » 13:00 — **Importance of nutrition in the oncologic patient: Nestlé Health Science nutritional solutions**
Marta Vasconcelos Nestlé Health Science, PT — *Sponsor lecture Nestlé*

13:00 » 15:00 — **Lunch, poster viewing** *Sala Colunata* **and Meet the Editor Maria Baratta (Editor of Cell Reports)** *Sala Templo, Hotel do Templo*

15:00 » 16:00 — **Closing lecture** — *EACR sponsored lecture*
Jacques Pouysségur Scientific Centre of Monaco, MC
Targeting acidic, nutritional and oxidative stresses in cancer

16:00 » 16:30 — **Best poster and oral communication award ceremony and closing session**
Fátima Baltazar ICVS, PT; **Ana Preto** CBMA, PT; **Joana Paredes** Vice-President of ASPIC; **Silvia Pastoreková** President of ISCaM

LOCAL ORGANIZING COMMITTEE

Ana Preto
University of Minho – PT
Centre of Molecular and Environmental Biology

Fátima Baltazar
University of Minho – PT
Life and Health Sciences Research Institute

Lúcio Lara Santos
Portuguese Institute of Oncology, Porto – PT

Julieta Afonso
University of Minho – PT
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Marta Costa
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University of Minho – PT
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Sara Granja
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SCIENTIFIC COMMITTEE

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Nicola Baldini
University of Bologna – IT
Department of Biomedical and Neuromotor Sciences

Paolo Porporato
University of Torino – IT

Pawel Swietach
University of Oxford – UK

Pierre Sonveaux
Université Catholique de Louvain, UCLouvain, BE

Silvia Pastorekova
Slovak Academy of Sciences in Bratislava – SK
Institute of Virology

Sofia Avnet
Istituto Ortopedico Rizzoli – IT
Orthopaedic Pathophysiology and Regenerative Medicine Lab

Stine Falsig Pedersen
University of Copenhagen – DK
Department of Cell and Developmental Biology

NOTES

Keynote Speakers & Abstracts

02

Jacques Pouyssegur

EDUCATION

Engineer in Biochemistry, 1966 INSA (University of Lyon)
Civil Military Prof. Agronomic Institute, Algiers, Algeria (1966-1968)
Doctor es-Sciences (Thesis) 1972 INSA (University of Lyon)
Post-doctorant National Cancer Institute (lab Ira Pastan), Bethesda, USA (1974-1976)
Sabbatical 1989, University San Francisco (lab. H. Bourne)
Sabbatical 1996, MIT (lab R. Weinberg)

WORK CAREER

Position
CNRS Research Director emeritus

Affiliation
University Cote d'Azur, UCA, IRCAN, Centre A. Lacassagne, Nice, FR; Department. Medical Biology Centre Scientifique de Monaco, CSM
Research Group Leader (1978-current) University Cote d'Azur, Nice,
CNRS Institutes (ISBDC, IRCAN)
Director of the CNRS Institute of Signaling, Development Biology and Cancer Research – (1997-2007)

Representative Awards
1989, Savoie Prize (LNCC); 1989, Delahautemaison Nephrology Prize (FRM); 1995, Rosen Cancerology Prize (FRM); 1996, Lounsbery Prize of American and French Academy of Sciences; 1999, Athena and Institut de France Prize; 2001, Leopold Griffuel Cancer Prize (ARC); 2002, Sir Hans Krebs Medal (FEBS); 2008, Carl Cori Lecture Award (Roswell Park, USA)

Member
EMBO; French Academy of Sciences; Europea Academy of sciences.

Research Areas of Interest
Control of cell division – Growth factors - Na+/H+ Antiporters – pH control - MAP kinases – Angiogenesis – Nutrient sensors – Hypoxia signaling – Tumour microenvironment – Metabolism and Cancer.

ABSTRACT

TARGETING ACIDIC, NUTRITIONAL AND OXIDATIVE STRESSES IN CANCER

I. Marchiq[1], M. Zdralevic[1], M.Vucetic[2], SK. Parks[2] and J. Pouysségur[1,2]
1. Institute for Research on Cancer and Aging, Nice (IRCAN) University of Nice, Centre A. Lacassagne, 33 avenue de Valombrose, Nice, France.
2. Medical Biology Department, Centre Scientifique de Monaco (CSM), Monaco.

In metazoans, sensing the availability of oxygen and key nutrients (glucose, amino acids, fatty acids) is integrated with growth factor and hormone signaling. This multiple nutrient and energy checkpoint converges on the activation of the master protein kinase TORC1, critical for engaging cells in the cell cycle and promoting growth. Cells have evolved sophisticated regulatory systems to rapidly respond to several lethal stressors including metabolic acidosis, nutritional depletion and reactive oxygen species. Cancer cells respond in multiple ways to escape and thrive these microenvironment stresses thus offering several strategies to combat cancer resilience before and after therapeutic treatment.

In this lecture we will discuss how we can exploit cancer vulnerabilities (metabolic tumor acidosis, amino acid depletion and oxidative stress) to propose novel anticancer targets capable to either arrest tumor growth or to kill cancer cells.

Diane L. Barber

EDUCATION

BS University of California Davis
MS University of California Davis
PhD University of California Los Angeles

WORK CAREER

Position
Endowed Professor and Chair
Department of Cell and Tissue Biology

Affiliation
University of California San Francisco

Representative Awards
Established Investigator American Heart Association
UCSF Innovation in Basic Sciences Award
Fellow, American Association for the Advancement of Science
Outstanding Faculty Mentoring Award, UCSF Postdoctoral Scholars Association
Faculty Research Lecturer Award
Chair, Women in Cell Biology (WICB) for American Society of Cell Biology (ASCB)

Research Areas of Interest
Intracellular pH regulation of protein dynamics from cancer to stem cell behaviors

ABSTRACT

Intracellular pH Regulation of Protein Dynamics: from Cancer to Stem Cell Behaviors

Katharine A. White, Bree Grillo-Hill and Diane L. Barber
University of California San Francisco

Introduction: Constitutively increased intracellular pH (pHi) is common to most cancers regardless of their tissue origin or genetic background. How a higher pHi promotes disease progression and the molecular mechanisms mediating pHi-dependent cancer cell behaviors, however, are poorly understood.

Experimental: We are determining how increased pHi enables multiple conserved features of cancers by bridging protein structural dynamics and cell biology to understand how protonation functions as a post-translational modification to regulate protein structure and function (Schönichen, 2013). Our multidisciplinary work includes the biochemistry of recombinant proteins, cell biology, and in silico computational analysis of protein dynamics as a function of tritrating amino acids.

Results: We have revealed in molecular detail the design principles and functions of selective pH-sensitive proteins regulating cancer cell behaviors, including cell metastasis, metabolic reprogramming, and tumorigenesis (Webb, 2011; Grillo-Hill, 2015; White, 2017a). Examples include GEFs regulating Cdc42 activity, cofilin, talin, focal adhesion kinase, and beta-catenin. Our recent work reveals how the higher pHi of cancer cells can enable the tumorigenic functions of charge-changing somatic mutations, particularly arginine to histidine mutations that we found are enriched in a subset of cancers (White, 2017b). We also found that increased pHi is necessary for the differentiation of stem cells (Ulmschneider, 2016), with relevance to cancer initiating cells, and we recently developed a genetically-encoded lysosome pH biossensor to determine how dysregulated lysosome pH in cancers can enable disease progression.

Conclusion: Our work is generating new views on how increased pHi promotes cancer behaviors with a focus protein dynamics enabling tumorigenic behaviors. Targeting the pH-regulated structure and function of selective proteins offers new therapeutic approaches to limit disease progression.

References:

Grillo-Hill, B.K., Choi, C., Jimenez-Vidal, M. and Barber D. L. 2015 Increased H+ efflux is sufficient to induce dysplasia and necessary for viability with oncogene expression. eLife 4:e03270.

Schönichen, A., Webb, B.E., Jacobson, M.P., and Barber, D.L. 2013 Considering protonation as a post-translational modification regulating protein structure and function. Ann Rev Biophys. 2013 42:289-314.

Ulmschneider, B., Grillo-Hill, B.K., Benitez, M., Azimova, D., Barber. D.L., Nystul, T.G. 2016 Increased intracellular pH is necessary for adult epithelial and embryonic stem cell differentiation. J. Cell Biol. 215:345-355.

Webb, B.E., Chimenti, M., Jacobson, M.P., and Barber, D.L. 2011 Dysregulated pH: a perfect storm for cancer progression. Nature Cancer Rev. 11:671-677.

White, K.A., Grillo-Hill, B.K., Barber, D.L. 2017a. Dysregulated pH dynamics enables cancer cell behaviors. J. Cell Sci. 130:663-669.

White, K.A., Garrido Ruiz, G., Szpiech, Z.A., Strauli, N.B., Hernandez, R.D., Jacobson, J.P. and Barber, D.L. 2017b Cancer-associated arginine to histidine mutations confer a gain in pH sensing to mutant proteins. Sci. Signaling 10(495). pii: eaam9931.

Invited Speakers & Abstracts

03

Ana M. Gil

EDUCATION

Ana Maria Gil (born 1965) obtained her Licenciatura in Chemistry at the University of Coimbra (1987) and her doctorate degree in Chemistry at the University of East Anglia, UK, in 1992. She has since been employed at the University of Aveiro, Portugal, where she is Associate Professor (since 1998) with "Habilitation" (since 2007).

WORK CAREER

Position
Associate Professor with "Habilitation"

Affiliation
University of Aveiro (UA), Department of Chemistry and CICECO - Aveiro Institute of Materials

Representative Positions
Several management positions at UA, e.g. presently CICECO coordinator of Research Line in Sustainability and Health.

Representative Awards
Several conference awards, sabbatical subsidies and funded projects.

Research Areas of Interest
Ana Gil's expertise ranges from solid-state nuclear magnetic resonance (NMR) and rheological-NMR to investigate biopolymer structure/function relationships, to more recent interests in metabolomics focusing on i) disease research, ii) drugs and biomaterials testing/development (with a present particular interest in stem cell metabolism) and iii) environmental (or exposome) impacts on human metabolism. She has published 125 SCI papers, 3 co-edited books, 25 book chapters, 30 proceedings and other papers, and presented more than 200 conference communications, including 49 invited lectures (37 abroad). Metrics (May 2019): h-index=38, 3,596 citations (no self-citations).

ABSTRACT

Metabolomics and the unveiling of new metabolic players in cancer

Ana M. Gil
Department of Chemistry and CICECO-Aveiro Institute of Materials

Metabolomics has extensively been employed in the search for disease biomarkers, by NMR and MS-based methods, either addressing cells, tissue or biofluids; either from human or animal model sources. Twenty years on since the first profiling study of human biofluids, much new knowledge has been gathered and, in tandem, many challenges have been recognized.
In this presentation, an initial account will be given of the use of biofluids in metabolomics, particularly those that may be collected (near) non-invasively (blood, urine). These may unveil systemic markers of cancer, but they also pose several important challenges, such as confounder variables, cohort size, inter-individual variability and biochemical interpretation. The usefulness of longitudinal designs and large metadata/sample banks will be discussed in a metabolomics context.

In the second part of the talk, attention will be given to the use of metabolomics, mainly NMR-based, to measure local tissue metabolic profiles, namely to evaluate tumour status, evolution, heterogeneity and response to therapy (including biotoxicity assessment in different organs). The underlying importance of tissue biochemical stability during handling will firstly be addressed, subsequently following onto some recent results obtained for animal models of pancreatic and breast cancers, to illustrate the issues of tumour heterogeneity and disease progression.

Aung Naing

EDUCATION

MD

WORK CAREER

Position
Associate Professor

Affiliation
Department of Investigational Cancer Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, Texas

Representative Positions
- Associate Member, The University of Texas Graduate School of Biomedical Sciences, Houston, Texas
- SWOG Early Therapeutic Committee, San Francisco, California
- Management of Immune-mediated Side Effects Guideline Panel, American Society of Clinical Oncology (ASCO), Chicago, Illinois
- SITC Immune Biomarkers Task Force, Society for Immunotherapy of Cancer (SITC), Milwaukee, Wisconsin
- Teaching Faculty, The SITC Cancer Immunotherapy Winter School, Mesa, Arizona

Representative Awards
- The ASCO Merit Award 2006, American Society of Clinical Oncology, 2006
- Fellow of The American College of Physicians, American College of Physicians, 2008-present
- Voted one of The Best Doctors in America, 2011-2012, 2013-2014, 2017-2018, 2019-2020

Research Areas of Interest
- Identify immunologic biomarkers to predict responders versus non-responders on immunotherapy-based clinical trials
- Identify predictive markers for immune-related adverse events (irAE) associated with immunotherapies
- Predict primary and secondary resistance to immunotherapy
- Develop immunotherapeutic strategies to overcome resistance

ABSTRACT

Targeting Metabolic Pathways in Cancer Therapy

Aung Naing
The University of Texas MD Anderson Cancer Center

Rapidly proliferating tumor cells have an increased metabolic demand. Cancer cells employ metabolic reprogramming to sustain survival and proliferation. As there is fierce nutrient competition between the tumor and the immune cells in the tumor microenvironment, metabolic pathways have been identified as therapeutic targets. Targeting these metabolic adaptations may thus be promising in the treatment of cancer. Among the several metabolic pathways that are being investigated, we present two pathways of interest: 1) arginine and 2) glutamine. Myeloid-derived suppressor cells (MDSCs) are immunosuppressive and extensive infiltration of MDSCs have been reported in solid tumors. These MDSCs express metabolic enzymes, such as arginase, which catalyzes the conversion of arginine to ornithine and urea. On stimulation, MDSCs release arginase that leads to local depletion of arginine in the tumor microenvironment. However, arginine is critical for proliferation and activation of cytotoxic T cells and natural killer cells. Thus, inhibition of arginase represents a promising therapeutic option. Glutamine is an important essential nutrient required for energy generation. Glutamine is deaminated to glutamate by glutaminases, which is then directed to Krebs cycle for energy production. However, many cancer cells overexpress glutaminase as they are glutamine dependent for cancer cell maintenance and growth. Further glutamine metabolism coordinates cancer cell signaling to promote tumor growth through mTOR pathway. Hence targeting glutamine metabolic pathway is a promising strategy.

Christian Frezza

Dr. Christian Frezza is Programme leader at the MRC Cancer Unit, Cambridge Cancer Center, at the University of Cambridge, UK. He studied Medicinal Chemistry at the University of Padova, Italy, and gained his MSc in 2002, after a period of research on mitochondrial toxicity induced by photoactivable anticancer drugs. Christian then joined the laboratory of Luca Scorrano in Padova to start a PhD on mitochondrial dynamics and apoptosis. In 2008, he moved to the Beatson Institute of Cancer Research in Glasgow as recipient of an EMBO Long Term Fellowship, where he investigated the role of mitochondrial defects in tumorigenesis. He moved to the MRC Cancer Unit in 2012 as tenure track Group Leader and became a Programme Leader in 2017.

His laboratory is mainly interested in investigating the emerging connection between cancer and metabolism, with a particular focus on mitochondrial metabolism. By using a combination of biochemistry, metabolomics, and systems biology he investigates the role of altered metabolism in cancer with the aim to understand how metabolic transformation regulates the process of tumorigenesis. His aim is to exploit these findings to establish novel therapeutic strategies and diagnostic tools for cancer.

Brief summary of the lines of research

Since starting my independent research group in 2012, I have pursued the emerging paradigm that dysregulated cellular metabolism can contribute, and in some cases, promote carcinogenesis. Our work focuses on the dysregulation of mitochondrial metabolism, which is emerging as a key feature of tumorigenesis with yet unclear molecular underpinnings. This work is the results of a natural progression of my longstanding interest in mitochondria, which started during my PhD with Luca Scorrano, where I contributed to a new understanding of the link between mitochondrial dynamics and apoptosis (Frezza et al Cell 2006), and later during my post docs with Eyal Gottlieb, where I laid the foundation of my current work on Fumarate Hydratase (Frezza et al Nature 2011). Our work has begun to contribute to a new understanding of mechanisms of tumour initiation and progression, which are invariably characterised by profound metabolic changes (Gaude et al, Nature Communications 2016), but whose role is only partially understood. Our recent work (Sciacovelli et al, Nature 2016) provided the first evidence that fumarate, a metabolite that accumulates in fumarate hydratase-deficient cancer cells, elicits the epigenetic suppression of a small non-coding RNA called miR200, triggering the epithelial-to-mesenchymal transition, an event critical for cancer initiation and metastasis. These findings demonstrate that metabolic reprogramming of cancer is not merely an epiphenomenon of cell transformation but, rather, that it can actively contribute to the activation of oncogenic signalling cascades. Recent work (Gaude et al, Mol Cell 2018) corroborated the relevance of dysregulated mitochondrial metabolism in driving oncogenic processes, capitalising on a unique model of mitochondrial DNA mutation. A key feature of our work is the combination of multidisciplinary approaches, from hard-core mitochondrial biochemistry, to systems biology, and metabolomics, and a unique network of collaborators. We also strive to apply our results to clinical relevant contexts.

ABSTRACT

Mitochondrial dysfunction and cancer

The role of mitochondrial dysfunction in cancer has been debated for over a century. Recent bioinformatic data analyses revealed that mitochondrial genes are suppressed in cancer with poor clinical outcome. Furthermore, the fact that mutations of core metabolic enzymes in the mitochondria such as Fumarate Hydratase (FH) cause renal cancer strongly indicates that mitochondrial dysfunction can drive cancer. Today, I will provide an overview of our recent findings about the molecular mechanisms through which mitochondrial dysfunction can drive transformation and shape cancer progression. In particular, using a novel genetically modified mouse model, I will show that FH loss has different outcomes in different tissues, and whilst the kidney are very robust to FH loss, other tissues don't tolerate FH loss and here FH-deficient cells are negatively selected. Our work provides some insights into potential mechanisms of tissue-specific tumorigenesis based on metabolic permissiveness.

Ciro Isidoro

EDUCATION

(1983) – Laurea Summa cum Laude - Doctor in Biological Sciences (D.Sc.), Università di Torino (Italy); (1999) - Laurea Summa cum Laude - Doctor in Medicine and Surgery (M.D.), Università del Piemonte Orientale (Novara, Italy)

WORK CAREER

Position
Associate (qualified Full) Professor of General Pathology

Affiliation
Università del Piemonte Orientale, Department of Health Sciences; via Paolo Solaroli 17 – 28100, Novara (Italy)

Representative Positions
Visiting Professor, Department of Cell Biology, Oklahoma University Health Science Center (OKC, US).
Co-Editor-in-Chief of J Traditional and Complementary Medicine;
Associate Editor of Autophagy, Molecular Carcinogenesis, BMC Cancer, Int J Molec Sci, Genes and Cancer, J. of Ovarian Research, J. Molecular Signaling
Member of the Scientific board of « Integrative Cancer Research Center of the Georgia Institute of Technology », Georgia Tech University, Georgia (Atlanta, US)
Executive Vice-President of the International Association of Traditional and Complementary Medicine.

Representative Awards
(2014) Professor Honoris Causa Faculty of Medicine and Pharmacy, Université de la Franche-Comté of Besançon (France).

Research Areas of Interest
Autophagy in Cancer and in Neurodegeneration. Epigenetic regulation of Autophagy and cell death. Anti-aging and anti-cancer Nutraceuticals. Organelle biogenesis, vesicular traffic and diseases. Nanotheranostics ('in cellulo' imaging).

ABSTRACT

IL-6 drives cancer cell EMT via glucose-dependent inhibition of autophagy

Chiara Vidoni[1], Alessandra Ferraresi[1], Letizia Vallino[1], Menaka Chinthakindi[1], Suyanee Thongchot[1], Eleonora Secomandi[1], Danny N Dhanasekaran[2] and Ciro Isidoro[1]
1. Laboratory of Molecular Pathology, Department of Health Sciences, Università del Piemonte Orientale (Novara, Italy);
2. Stephenson Cancer Center, OUHSC (Oklahoma City, OK, US)

Epithelial-to-Mesenchymal Transition (EMT) refers to the ability of cancer cells to reprogram their morphogenetic and functional state to adapt to survive and move within the extracellular matrix. This phenotypic change is reversible, it is under epigenetic control, and is associated with changes in the metabolism of glucose. Autophagy, a cellular degradation process induced as stress response to glucose depletion, opposes to cancer cell EMT and migration. Inflammatory cytokines, such as IL-6 and IL-8, promotes EMT. We found that IL-6 induces EMT and invasivenes of cancer cells via stimulating the uptake of glucose and the inhibition of autophagy. We show that inhibition of the PI3KC1-AKT pathway limits the GLUT-mediated uptake of glucose and this results in the HEK-II inhibition of mTOR and consequent activation of autophagy, which concurs to arrest cancer cell migration and eventually reverts the mesenchymal-like phenotype into epithelial-like phenotype.

Cristina Muñoz Pinedo

EDUCATION

PhD in Biology, Universidad de Granada

WORK CAREER

Position
Group Leader of the "Cell Death Regulation Group"

Affiliation
Oncobell Program, Bellvitge Biomedical Research Institute

Representative Positions
1997 - 2001 PhD student. Spanish National Research Council (CSIC), Granada, Spain
1998 Visiting student, University College London, UK (Sep – Oct 1998)
1999 Visiting student, Institut Gustave Roussy. CNRS. Villejuif (France) (Sep - Nov 1999).
2000 Visiting student, La Jolla Institute for Allergy and Immunology, CA, USA (Aug-Dec 2000).
2002 - 2005 Postdoctoral researcher, La Jolla Institute for Allergy and Immunology, CA, USA
2006 Postdoctoral associate, St. Jude Children's Research Hospital, Memphis (Tennessee), USA
2006 - 2012 "Miguel Servet" Investigator, IDIBELL, Spain, 2006-2012.
2012 - Present Group leader, IDIBELL, Spain

Other Experience and Professional Memberships
2017 Member of the Advisory Board of the FEBS Journal
2015 Editorial Board member, Oncogenesis
2014 Editorial Board member, Molecular & Cellular Oncology
2011 Handling editor, Cell Death and Disease

Research Areas of Interest
Cristina Muñoz-Pinedo's group researches the mechanisms through which tumour cells die in response to different treatments, and to glucose metabolism inhibition in particular. The lab studies starvation responses at the cellular level and their influence on the tumour microenvironment, including the immune system.

ABSTRACT

Online publication not authorized.

Fátima Martel

EDUCATION

Degree in Biology (Scientific Domain) by the Faculty of Sciences of the University of Porto, in 1989
PhD in Human Biology by the Faculty of Medicine of the University of Porto, in 1995
Habilitation ("Agregação") in Metabolism by the Faculty of Medicine of the University of Porto, in 2009

WORK CAREER

Position
Associated Professor, since 2005

Affiliation
FMUP (Faculty of Medicine of University of Porto)

Representative Positions
member of the Scientific Committee of the Doctoral Programmme of FMUP in Metabolism-Basic and Clinical investigation

Representative Awards
Honour mention in "Prémio da Boa Esperança" (1994); 2º Prize UCB of Pharmacology (1996); Prize "Gonçalves Ferreira de Nutrição/Alimentação and Prize Ricardo Jorge de Saúde Pública" (2001); Nutrition Award from the "Associação Portuguesa de Nutricionistas" (2010)

Research Areas of Interest
Epitelial (placental, intestinal, etc) plasma membrane transport of organic compounds (vitamins (folic acid, thiamine), organic cations, catecholamines, serotonin, butyrate, glucose, L-methionine, L-alanine); plasma membrane transport of nutrients in cancer cells; modulation of transport by polyphenols and other food constituents, drugs of abuse and pathological conditions.

ABSTRACT

Effect of dietary polyphenols on nutrient uptake: a new potential therapeutic target for cancer

Fátima Martel[1,2]
1. Unit of Biochemistry, Department of Biomedicine, Faculty of Medicine, University of Porto, Porto, Portugal
2. Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

Dysregulated metabolism to support high proliferation rates is now recognized as a hallmark of cancer cells. A key alteration in energy metabolic pathways in cancer cells, whereby the rate of glucose uptake is significantly increased and a high rate of glycolysis and lactate production occurs even when oxygen is present ("aerobic lactatogenesis"), is known as the Warburg effect. Interestingly, an intratumoral energetic symbiosis, involving lactate recycling, has been described, as oxygenated tumor cells prefer to use lactate as an oxidative fuel, allowing glucose to diffuse farther from blood vessels where it is taken up by glycolytic tumor cells, in turn producing lactate in large quantities. Accordingly, GLUT1 and MCT1, which are the main glucose and lactate transporters in cancer cells, respectively, are seen as oncogenes and as potential therapeutic targets in cancer treatment.

Polyphenols, commonly contained in fruits and vegetables, have a beneficial action in the prevention of diseases associated with oxidative stress, such as cancer. Recent evidence suggests that interference of polyphenols with nutrient cellular uptake is a mechanism contributing to their protective role against cancer. Specifically for breast cancer, in vitro studies show that several polyphenols inhibit glucose transport, and an association between inhibition of glucose cellular uptake and their anticarcinogenic effect was found. Moreover, some polyphenols are able to inhibit lactate uptake and some are able to inhibit both glucose and lactate uptake, thus depleting breast cancer cells of their two most important energy suppliers. Finally, cancer cells also possess an increased need of glutamine ("glutaminolysis") and some polyphenols were recently found to interfere with the cellular uptake of glutamine by breast cancer cells.

So, the antimetabolic effect of polyphenols should be regarded as a mechanism of action contributing to their chemopreventive/chemotherapeutic potential.

Acknowledgements: Fundação para a Ciência e a Tecnologia (UID/BIM/04293/2013).

Fatima Mechta-Grigoriou

EDUCATION

PhD

WORK CAREER

Position
Deputy Director "Genetic and Biology of Cancer" Unit, head of "Stress and Cancer" laboratory

Affiliation
Institut Curie, Inserm

Representative Positions
DR1 Inserm

Representative Awards
EMBO member

Research Areas of Interest
Oxidative stress, Metabolism, Stroma, Breast Cancers, Ovarian Cancers

ABSTRACT

Role of oxidative stress and stromal heterogeneity in Cancer

Fatima MECHTA-GRIGORIOU
Institut Curie, Stress and Cancer laboratory, U830 Inserm, 25 rue d'Ulm, 75005 Paris, France

In last years, we have investigated the pathophysiological consequences of a faint but chronic oxidative stress. We demonstrated that a chronic oxidative stress increases metastatic spread by deeply modifying stroma (EMBO Mol Med, 2010; Semin Cancer Biol, 2014; Oncogene, 2017; ARS, 2017).

In the meantime, we showed that a chronic stress can improve response to chemotherapy. This was first observed in high-grade serous ovarian cancer (HGSOC) in response to Taxanes (Nature Medicine, 2011). We identified the first molecular mechanism driven by the miR-200 that leads to two molecular subgroups (Stress / Fibrosis – Low / High-OXPHOS) of HGSOC patients with different survival (IJBCB, 2014; Nat Commun, 2016; Cell metabolism 2019). Furthermore, we deciphered that MEK/ERK signaling is up-regulated in high-MAP3K8 HGSOC, suggesting that MEK inhibitors could be useful for HGSOC treatment (Nat Commun, 2015). Importantly, the positive impact of oxidative stress on chemosensitivity was also observed in breast cancers through 2 distinct mechanisms, autophagy (Autophagy, 2014) and DNA damage response (EMBO Mol Med, 2016).

Finally, by studying 6 stromal markers concomitantly and combining various approaches, we identified 4 CAF subsets (CAF-S1 to CAF-S4) in breast cancers (Cancer Cell, 2018). We confirmed their existence in ovarian cancers (Nat. Commun, 2018), showing the relevance of our findings in distinct cancer types. We found that CAF-S1 fibroblasts promote immunosuppression through a multi-step mechanism. CAF-S1 attract T CD4+CD25+ T lymphocytes, enhance their survival, stimulate their differentiation into CD25HighFOXP3High and promote the capacity of regulatory T cell to inhibit T effector proliferation. We uncovered several molecules, expressed by CAF-S1 fibroblasts, which are involved in the different steps of the CAF-S1-mediated immunosuppressive activity (Cancer Cell, 2018; Nat. Commun, 2018).

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Batista L, Nature Communications. 2016 Jan 4;7:8959.
Gruosso T, Nature Communications. 2015. Oct 12;6:8583.
Lefort S, Autophagy. 2014. Dec 2;10(12):2122-42.
Mateescu B, Nature Medicine. 2011, 17(12):1627-35

Jacinta Serpa

EDUCATION

Azores University, Portugal | MSc | December/1997 | Applied Biology
Medical Faculty, Oporto University, Portugal | PhD | December/2005 | Human Biology

WORK CAREER

Position
Assistant Professor

Affiliation
Centro de Estudos de Doenças Crónicas da NOVA Medical School (CEDOC|NMS); Instituto Português de Oncologia de Lisboa, Francisco Gentil (IPOLFG)

Representative Positions
- Since October 2013- Invited researcher in Instituto Português de Oncologia de Lisboa, Francisco Gentil
- Since September 2008 - Assistant Professor of Pathology in NOVA Medical School
- January 2006 to September 2013- Post- doc investigator in Angiogenesis group from CIPM-IPOLFG-BPD (FCT)
- January 2002 to December 2005- Development of PhD thesis "SECRETOR AND LEWIS PHENOTYPE/ GENOTYPE AND THEIR RELEVANCE FOR HELICOBACTER PYLORI INFECTION" in IPATIMUP- BD grant (FCT)
- June 1999 to October 2000- Production of recombinant fusosyltransferase III in Sf9 insect cells with the baculovirus system- BIC grant (FCT)
- January 1998 to May 1999- Production of peroxidases from plant origin in INETI-JTI grants (PEDIP)
- February 1997 to December 1997- Production of monoclonal antibodies and recombinant antibodies and enzymes in INETI- PRODEP

Representative Awards
Liga Portuguesa Contra o Cancro (LPCC) Prize; 2008

Research Areas of Interest
Jacinta Serpa has a PhD in Human Biology by The Medical Faculty of Oporto University (2005) and Graduated in Applied Biology by The Azores University (1997).
She is an expert in cancer biology with more than 20 years of experience and 25 peer reviewed published papers. Her main field of interest is cancer metabolism, allowing cancer cells survival in a certain microenvironment and also the way endogenous cancer metabolism interferes with cell response to drugs. Since, 2016 she is the head of Cancer Metabolism and Microenvironment Lab in CEDOC|NMS (http://cedoc.unl.pt/cancer-metabolism-and-microenvironment/). She participated in more than 15 research projects and she has supervised 11 Master thesis and 5 PhD Thesis.

ABSTRACT

Cancer cells metabolic heterogeneity underlies chemoresistance

Sofia C Nunes[1,2], Filipa Lopes-Coelho[1,2], Lidia S Silva[2], Sofia A Pereira[1], João B Vicente[3], Luis G Gonçalves[3] and Jacinta Serpa[1,2]
1. Centro de Estudos de Doenças Crónicas da NOVA Medical School (CEDOC|NMS);
2. Instituto Português de Oncologia de Lisboa, Francisco Gentil (IPOLFG);
3. Instituto de Tecnologia Quimica e Biológica, António Xavier (ITBQ-NOVA);

The metabolic remodeling not only allows the establishment of a tumor in a certain microenvironment, but it also permits resistance to therapy. Within a tumor, a range of different metabolic profiles can be found amongst cancer cells. This cell heterogeneity contributes for a stronger cell population capable of resisting to stressful conditions, such as hypoxia and drugs exposure.
The establishment of metabolic symbiosis is a strategy developed by tumors and positively selected by microenvironment to successfully carry on disease progression.
Different cancer models will be addressed under this topic in order to show the vast adaptability of cancer cells in order to benefit from different microenvironments.

References:

Nunes SC, Ramos C, Lopes-Coelho F, Sequeira CO, Silva F, Gouveia-Fernandes S, Rodrigues A, Guimarães A, Silveira M, Abreu S, Santo VE, Brito C, Félix A, Pereira SA, Serpa Cysteine allows ovarian cancer cells to adapt to hypoxia and to escape from carboplatin cytotoxicity. Sci Rep. 2018 Jun 22;8(1):9513. doi: 10.1038/s41598-018-27753-y.

Nunes SC, Lopes-Coelho F, Gouveia-Fernandes S, Ramos C, Pereira SA, Serpa J. Cysteine boosters the evolutionary adaptation to CoCl2 mimicked hypoxia conditions, favouring carboplatin resistance in ovarian cancer. BMC Evol Biol. 2018 Jun 19;18(1):97. doi: 10.1186/s12862-018-1214-1.

Lopes-Coelho F, Nunes C, Gouveia-Fernandes S, Rosas R, Silva F, Gameiro P, Carvalho T, Gomes da Silva M, Cabeçadas J, Dias S, Gonçalves LG, Serpa J. Monocarboxylate transporter 1 (MCT1), a tool to stratify acute myeloid leukemia (AML) patients and a vehicle to kill cancer cells. Oncotarget. 2017 Aug 16;8(47):82803-82823. doi: 10.18632/oncotarget.20294.

Lopes-Coelho F, André S, Félix A, Serpa J. Breast cancer metabolic cross-talk: Fibroblasts are hubs and breast cancer cells are gatherers of lipids. Mol Cell Endocrinol. 2018 Feb 15;462(Pt B):93-106. doi: 10.1016/j.mce.2017.01.031.

Silva LS, Goncalves LG, Silva F, Domingues G, Maximo V, Ferreira J, Lam EW, Dias S, Felix A, Serpa J. STAT3:FOXM1 and MCT1 drive uterine cervix carcinoma fitness to a lactate-rich microenvironment. Tumour Biol. 2016 Apr;37(4):5385-95. doi: 10.1007/s13277-015-4385-z.

Lopes-Coelho F, Gouveia-Fernandes S, Gonçalves LG, Nunes C, Faustino I, Silva F, Félix A, Pereira SA, Serpa J. HNF1β drives glutathione (GSH) synthesis underlying intrinsic carboplatin resistance of ovarian clear cell carcinoma (OCCC). Tumour Biol. 2016 Apr;37(4):4813-29. doi: 10.1007/s13277-015-4290-5.

Javier A. Menendez

EDUCATION

1997 Bachelor's Degree in BIOLOGICAL SCIENCES (B. Sc.), Oviedo University, Asturias, Spain
2000 Master of Science in BIOCHEMISTRY and MOLECULAR BIOLOGY (M. Sc.), Complutense University, Madrid, Spain
2001 Doctor Degree in BIOCHEMISTRY and MOLECULAR BIOLOGY (Ph. D.), Complutense University, Madrid, Spain
2015 "Ad Honorem" Professor, Department of Medicine and Surgery, Rovira i Virgili University (Reus, Spain)

WORK CAREER

Position
Group Leader, Metabolism & Cancer Group

Affiliation
Program Against Cancer Therapeutic Resistance (ProCURE), Catalan Institute of Oncology-Girona Biomedical Research Institute, Spain

Representative Positions
1997-2001 PRE-DOCTORAL FELLOW, Division of Medical Oncology, Hospital Universitario 12 de Octubre, Madrid, Spain
2001-2002 BIOLOGIST VISITING POST-DOCTORAL FELLOW, Life Science Division (LSD), Lawrence Berkeley National Laboratory (LBNL), University of Berkeley, California, USA
2002-2003 RESEARCH ASSOCIATE, Department of Medicine, Evanston Northwestern Healthcare (ENH), Breast Cancer Translational Research Program, ENH Research Institute (ENHRI), Evanston, Illinois, USA
2003-2006 ASSISTANT PROFESSOR, Department of Medicine, Division of Hematology/Oncology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA
2004-2005 SCIENTIST, Department of Medicine, Evanston Northwestern Healthcare (ENH), Breast Cancer Translational Research Program, ENH Research Institute (ENHRI) Evanston, Illinois, USA
2005-2006 FULL MEMBER of the Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, Illinois, USA
2006-2007 "MIGUEL SERVET" RESEARCH SCIENTIST, Girona Biomedical Research Institute (IDIBGI), Girona, Catalonia, SPAIN
2007-2010 STAFF SCIENTIST, Catalan Institute of Oncology (ICO), Girona, Catalonia, SPAIN
2010-Present TENURE, Catalan Institute of Oncology (ICO), Girona, Catalonia, SPAIN
2016-2018 Co-Founder & Chairman of METABOSTEM –Metabolic Drugs for Cancer Stem Cells-

Representative Awards
2001 Awards on Food, Nutrition and Health, INSTITUTO DANONE (Barcelona, SPAIN)
2004 Best Research CAREER DEVELOPMENT AWARD from the: Specialized Program of Research Excellence –SPORE- in Breast Cancer, NATIONAL CANCER INSTITUTE USA –NCI USA-
2004 & 2006 Awards for Basic, Clinical and Translational Research from the: SUSAN G. KOMEN BREAST CANCER FOUNDATION, USA
2005 Best Research CAREER AWARD (Best Young Investigator of the Mid-West in the USA) from the: Robert H. Lurie Comprehensive Cancer Center (Chicago, USA)
2005 BEST IDEAS AWARD from the medical magazine DIARIO MEDICO (Spain) in the Research and Pharmacology category
2006 "ANNALS of ONCOLOGY Award" for the Best Article in Translational Research
2008 BEST IDEAS AWARD from the medical magazine CORREO FARMACÉUTICO (Spain)
2010 The first "OLIVE OIL AND HEALTH" Research Award from the: Jaén Rural Savings Bank Foundation (Fundación Caja Rural de Jaén, Spain)
2011 Best Young Investigator Award (for those <40 years of age) from the: Girona Biomedical Research Institute (IDIBGI)-Catalan Research Centers of Excellence
2016 FUJITSU-INNOMEDYX Award, Best Innovative Idea. Girona Biomedical Research Institute (IDIBGI)
2018 IV Edition of Castillo de Canena LUIS VAÑÓ Award, Research on Olive Cultivation and Olive Oil: UC Davis Olive Center, Castillo de Canena, and Universidad de Jaén

Research Areas of Interest
Cancer; aging; metabolism; mitochondria; stem cells; epigenetics; natural biocompounds; nutraceuticals; new therapeutics

ABSTRACT

Metformin: a metabolo-epigenetic drug for cancer therapy

Javier A. Menendez
Metabolism & Cancer Group, Program Against Cancer Therapeutic Resistance (ProCURE), Catalan Institute of Oncology-Girona Biomedical Research Institute, Spain

The biguanide metformin, more than 60 years after its introduction as a first-line therapeutic for managing type 2 diabetes, is rapidly emerging as an archetypal compound capable of targeting the metabolism-epigenome axis in human malignancies. Intriguingly, such metabolo-epigenetic facet of metformin as anti-cancer therapy appears to operate in multi-faceted ways. First, downstream of its primary inhibitory action on cellular bioenergetics, metformin can indirectly alter the abundance of mitochondrial metabolites that are substrates and cofactors of chromatin-modifying enzymes. Second, metformin can shape the functioning of key metabolic enzymes whose activities are directly linked to changes in chromatin and DNA epigenetic states. Third, metformin can directly target the activity of core chromatin modifiers of the epigenome. This talk will summarize the most recent evidence collected in our laboratory unraveling unforeseen in vitro and in vivo metabolo-epigenetic effects of metformin including the inhibition of a central "charging" reaction of the folate cycle, namely the conversion of serine to glycine by the mitochondrial serine hydroxymethyltransferase 2 (SHMT2) enzyme, or altering the arginine-to-citrulline metabolism not only as part of the urea cycle but also likely as part of the protein arginine methylation and citrullination processes involved in epigenetic regulation. The goal is now to combine this information to generate a map of metformin-driven histone and DNA modifications to ultimately decipher the metabolo-epigenetic "language" of metformin in cancer diseases.

Marina Kreutz

EDUCATION

1999 Graduation as "Privatdozent" ("Habilitation") University of Regensburg, Venia legendi (Lecturer for "Experimental Hematology")
1988-1992 PhD thesis, University of Freiburg (Prof. Dr. R. Andreesen) "Induction of human monocyte differentiation by Vitamin D3"
1986-1987 Diploma thesis at the Institute for Immunobiology, University of Freiburg (Prof. Dr. W. Bessler) "Activation of murine macrophages by bacterial components: induction of interleukin-1 secretion by Tripalmitoylpentapeptide, a synthetic lipoprotein"
1980-1986 Albert-Ludwigs-University Freiburg, Diploma in Biology

WORK CAREER

2012 - Present Professor, Molecular Oncology, Dept. of Internal Medicine III
2000 Assistant Professor at the Dept. of Hematology and Oncology
1996 Group leader at the Dept. of Hematology and Oncology, University of Regensburg
1995 Research fellow at the University of California San Diego/UCSD (Prof. Dr. C. Glass)
1991-1994 Research scientist at the University of Regensburg

Affiliation

University Hospital Regensburg, Department of Internal Medicine III-Molecular Oncology
Franz-Josef-Strauss-Allee 11, D-93053 Regensburg

Research Areas of Interest

Modulation of immune cells by the tumor environment

- Immunometabolism
- Role of 1alpha,25-dihydroxyvitamin D3 for immune cell differentiation

Five most important publications

1. Brand A, Singer K, Koehl GE, Kolitzus M, Schoenhammer G, Thiel A, Matos C, Bruss C, Klobuch S, Peter K, Kastenberger M, Bogdan C, Schleicher U, Mackensen A, Ullrich E, Fichtner-Feigl S, Kesselring R, Mack M, Ritter U, Schmid M, Blank C, Dettmer K, Oefner PJ, Hoffmann P, Walenta S, Geissler EK, Pouyssegur J, Villunger A, Steven A, Seliger B, Schreml S, Haferkamp S, Kohl E, Karrer S, Berneburg M, Herr W, Mueller-Klieser W, Renner K, Kreutz M. (2016) LDHA-Associated Lactic Acid Production Blunts Tumor Immunosurveillance by T and NK Cells. Cell Metab. Nov 8;24(5):657-671
2. Kreutz M, Karrer S, Hoffmann P, Gottfried E, Szeimies RM, Hahn J, Edinger M, Landthaler M, Andreesen R, Merad M, Holler E (2012). Whole-Body UVB Irradiation during Allogeneic Hematopoietic Cell Transplantation Is Safe and Decreases Acute Graft-versus-Host Disease. J. Invest. Dermatol. Jan;132(1):179-87
3. Fischer K, Hoffmann P, Voelkl S, Meidenbauer N, Ammer J, Edinger M, Gottfried E, Schwartz S, Rothe G, Hoves S, Renner K, Timischl B, Mackensen A, Kunz-Schughart L, Andreesen R, Krause SW, Kreutz M (2007) Inhibitory effect of tumor cell derived lactic acid on human T cells. Blood 109:3812-3819
4. Gottfried E., Kunz-Schughart L., Ebner S, Mueller-Klieser W, Hoves S, Andreesen R, Mackensen A, Kreutz M (2005). Tumor-derived lactic acid modulates dendritic cell activation and antigen expression. Blood 107:2013-2
5. Fritsche J, Mondal K, Ehrnsperger A, Andreesen R, Kreutz M (2003), Regulation of 25-hydroxyvitamin D3-1alpha-hydroxylase and production of 1alpha25-dihydroxyvitamin D3 by human dendritic cells. Blood 102:3314-3316

ABSTRACT

Lactate: a metabolic immune checkpoint in the tumor environment

Marina Kreutz, Katrin Singer and Kathrin Renner
Department of Internal Medicine III and Regensburg Center for Interventional Immunology, University of Regensburg, Regensburg, Germany

Introduction: Accelerated glucose metabolism, known as "Warburg effect", is a hallmark of cancer cells and associated with upregulation of lactate dehydrogenase A (LDHA) and monocarboxylate transporters (MCTs). MCTs co-transport lactate and protons, which leads to "lactic acid" accumulation in the tumor environment. High levels of lactate correlate with incidence of distant metastases and elevated LDHA expression is associated with poor outcome in tumor patients. Based on these data, increased glycolysis by tumor cells appears to promote tumor growth and metastasis. We suggest that lactate and acidification support tumor progression via local immunomodulation.

Experimental: We studied the impact of lactic acid on different types of human and murine immune cells in vitro and in immunocompetent and immunodeficient C57BL/6 mice. Furthermore we blocked lactate secretion and analyzed its impact on checkpoint therapy.

Results: Lactic acid inhibited the activation and differentiation of human immune cells and prevented upregulation of nuclear factor of activated T cells (NFAT) in murine T and NK cells resulting in diminished IFN-□ production in vitro. In vivo, LDHA-associated lactic acid accumulation in melanomas inhibited tumor surveillance by T and NK cells. Tumors with reduced lactic acid production (Ldhalow) developed significantly slower than control tumors, and showed increased infiltration with IFN-□-producing T and NK cells in C57BL/6 mice.
We identified diclofenac as a potent inhibitor of the main lactate transporters MCT 1 and 4. Diclofenac diminished lactate secretion in all malignant cells. Based on these observations we investigated whether a pharmacologic blockade of lactate release by diclofenac could support checkpoint therapy. Indeed, co-administration of diclofenac but not aspirin improved the response to anti-CTLA4 and anti-PD1 therapy in a murine tumor model.

Conclusion: Our results demonstrate that lactic acid is a potent inhibitor of function and survival of T and NK cells leading to tumor immune escape.

References:

Brand A, Singer K, Koehl GE, ... Renner K, Kreutz M (2016) LDHA-Associated Lactic Acid Production Blunts Tumor Immunosurveillance by T and NK Cells. Cell Metab. Nov 8;24(5):657-671
Fischer K, Hoffmann P, Voelkl S, ... Krause SW, Kreutz M (2007) Inhibitory effect of tumor cell derived lactic acid on human T cells. Blood 109:3812-3819
Gottfried E., Kunz-Schughart L., Ebner S, ..., Mackensen A, Kreutz M (2005). Tumor-derived lactic acid modulates dendritic cell activation and antigen expression. Blood 107:2013-2

Marja Jäättelä

EDUCATION

MD 1989, PhD 1990

WORK CAREER

Position
Professor, Head of the Cell Death and Metabolism Unit

Affiliation
Danish Cancer Society Research Center

Representative Awards
Novo Nordisk Prize for Excellence in Biomedical Research
Suffrage Science Award
Descartes Prize for Research Collaboration, European Commission
Elected member of European Molecular Biology Organization (EMBO)
Elected member of the Royal Academy of Sciences, Denmark
Elected member of the Finnish Academy of Science and Letters

Research Areas of Interest
- Cancer
- Lysosomes
- Lipids and membranes
- Alternative cell death pathways

ABSTRACT

Lysosomal control of cytosolic pH

Bin Liu and Marja Jäättelä
Cell Death and Metabolism and Center for Autophagy, Recycling and Disease Danish Cancer Society Research Center, Copenhagen, Denmark

In spite of their acidic environment and increased acid production, cancer cells maintain alkaline intracellular pH, which is essential for their malignant growth. We have identified Signal Transducer and Activator of Transcription 3 (STAT3), which is best known for its cancer-promoting transcriptional activity, as a key player in the preservation of alkaline cytosol in cancer cells. A small pool of STAT3 associates with the lysosomal proton pump and increases its activity. Cytosolic acidification by various means further promotes the lysosomal localization and activity of STAT3 while inhibiting STAT3-dependent transcription. Our data reveal STAT3 as an essential regulator of intracellular pH, and vice versa intracellular pH as a potent regulator of STAT3 localization and activity. Furthermore, these data indicate that lysosomes, in spite of their relatively small volume, play an important role in the maintenance of the alkaline cytosol in cancer cells. Here, I will discuss possibilities to target this lysosomal function in future cancer therapy.

Massimiliano Mazzone

EDUCATION

2006 - 2009 Postdoctoral Fellow, Vesalius Research Center, VIB, Catholic University of Leuven (KU Leuven), Belgium
2002 - 2007 PhD in Cell Science and Technology, Division of Molecular Oncology, IRCC, School of Medicine, University of Torino, Italy.
1997 - 2002 Master (5-year course) in Medical Biotechnology (full marks and exceptional honors), School of Medicine, University of Torino, Torino, Italy.
1992 - 1997 Scientific Lyceum (60/60 first class honors), Institute Marie Curie, Pinerolo, Torino, Italy.

WORK CAREER

Position

04/2019 - Present Guest Full Professor, Molecular Biotechnology Center, University of Torino (IT)
10/2017 - Present Full Professor (permanent position), Department of Oncology, KU Leuven, Leuven (BE)
10/2009 - Present Group Leader, Laboratory of Tumor Inflammation and Angiogenesis
VIB Center for Cancer Biology (CCB), Leuven (BE)

Affiliation

VIB Center for Cancer Biology (CCB), Leuven (BE) – KU Leuven

Representative Positions

10/2014 - 09/2017 Associate Professor (permanent position), Department of Oncology, KU Leuven (BE)
10/2009 - 09/2014 Tenure Track, Assistant Professor, Department of Oncology, KU Leuven (BE)
11/2006 - 09/2009 Postdoctoral fellow, Prof. P. Carmeliet Lab, VIB-KU Leuven (BE) - supported by an EMBO-Long Term Fellowship

Representative Awards

ERC Consolidator Grant (2017)
ERC Proof-of-Concept (2016)
Award Foundation AstraZeneca (2015)
Chiara D'Onofrio Award, Senior category (2015)
EMBO Young Investigator Award (2014)
André Vander Stricht-Emile Carpentier Prize, King Baudouin Foundation (2014)
ERC Starting Grant (2012)
Lorini Award Foundation Andrea and Libi Lorini (2011)

Research Areas of Interest

Cancer, Cardiovascular medicine, Hypoxia, Tumor and inflammation, Macrophages, Angiogenesis, Immunoregulation

ABSTRACT

Harnessing tumor metabolism to overcome immunosuppression

Ricardo Amorim, Mathieu Pecqueux, Chiara Varamo and Massimiliano Mazzone
VIB Center for Cancer Biology - KULeuven

Introduction: Cancer immunotherapies, including immune checkpoint blockers (ICB) such as α-PD-1, α-PD-L1 or α- CTLA-4 antibodies, have provided patients with a promising treatment option. However, some tumors such as pancreatic ductal adenocarcinoma (PDAC) fail to show any clinical benefit. This is mostly due to low infiltration of cytotoxic T cells and to the immunosuppressive features of the tumor microenvironment (TME), altogether impeding the capacity of effector cells to survive, proliferate and tackle the tumor, thus contributing to immunological tolerance and limited success of this therapeutic option.

Experimental: Cytidine deaminase (CDA), an enzyme of the pyrimidine salvage pathway was recently identified in our lab as potential target involved in unresponsiveness to ICB. By knocking out CDA in pancreatic cancer cell lines, we address how the absence of CDA per se or in combination with ICB, impinges on tumor growth and on the remodeling of the immune landscape.

Results: Our results show that genetic inactivation of CDA in cancer cells decreases tumor growth, synergizes with α-PD-1 treatment and increases the recruitment and activation of cytotoxic T cells into the tumor. This was ascribed to an impairment of the glyco-coat in CDA KO cells because of a reduction in UDP-lined glycosyl blocks, which leads to an ER-stress response. The ultimate result is that CDA KO cancer cells have increased immunogenicity and are better recognized by the immune system, which improves the anti-tumor immune response.

Conclusion: These findings provide novel evidence that CDA inhibition in pancreatic tumors can modulate the TME, in particular by recruiting cytotoxic T cells and consequently improve the response of those tumors that are highly resistant to ICB.

Pawel Swietach

EDUCATION

WORK CAREER

Position

Affiliation

Representative Positions

Representative Awards

Research Areas of Interest

ABSTRACT

Acidity is a chemical signature of tumors and an important selection pressure that has the potential to change the phenotypic landscape of cancer tissue. Due to mutations, cancer cells are inherently diverse in their genetics, which yields considerable heterogeneity in phenotype. Since phenotype is ultimately the subject to selection pressures, it is important to understand the acid sensing and acid handling behaviours of cancer cells. To address this, we use a panel of 100+ colorectal cancer cells that have been carefully characterised in terms of mutations and gene expression. Now, we are measuring pH-related phenotypes with high throughput methods, in order to correlate these with gene expression patterns. Thus, we are able to describe genes that may underpin specific categories of cell behaviours, and potentially new targets which may be exploited to change the trajectory of the disease. My talk will describe our approach and present some preliminary data. I will also discuss whether it is appropriate to consider a single cell as the unit of selection in the process of somatic evolution.

Sarah Halford

EDUCATION

1984 - 1987 MA Physiological Sciences Oxford University
1990 MBBS University of London
1994 MRCP
1999 – 2003 Translational Clinical Research Fellow - Molecular and Population Genetics Laboratory, London; Cancer and Immunogenetics Laboratory, Oxford: Cancer Research UK – PhD University of London
Fellow of the Royal College of Physicians
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WORK CAREER

Position

Head of Medical Sciences Centre for Drug Development Cancer Research UK
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Affiliation

Cancer Research UK
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Research Areas of Interest

Early Phase Clinical Trials – Preclinical and clinical development of novel anticancer agents

ABSTRACT

Remove The Road Blocks: - A multi-disciplinary approach for the development of the first-in-human, first-in-class monocarboxylate transporter 1 (MCT1) inhibitor, AZD3965, targeting patients with advanced solid tumours and DLBCL

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Introduction: A key metabolic alteration in tumour cells is increased dependency on glycolysis, resulting in the production of lactate which is transported out of cells by MCTs. Inhibition of MCT-1 can constrain cancer cell growth in preclinical models. We report results on the phase I study of AZD3965, a FIC inhibitor of MCT-1.

Experimental: Patients with advanced solid tumours were treated with oral (po) AZD3965 at total daily doses of 5-30mg given once (od) and twice daily (bd). Exclusion criteria included a history of retinal or cardiac disease due to preclinical toxicology findings in the eye and heart (which express MCT-1). The primary objectives were to determine the safety, dose limiting toxicities (DLT) and maximum tolerated dose (MTD) of AZD3965. Intensive pharmacokinetic (PK) profiling was performed with subsequent modelling for receptor occupancy. Pharmacodynamic profiling included imaging to detect pH changes and tumour glucose uptake, and plasma/urine metabolomics.

Results: 40 patients (24M:16F; median age 64.5 were treated at dose levels of 5, 10, 20, and 30mg od and 10 and 15mg bd. AZD3965 was well tolerated with nausea and fatigue (CTCAE Gr1-2) the most commonly reported side effects. A single DLT of cardiac troponin rise was observed at 20mg od. Asymptomatic, reversible retinal ERG changes were first observed at 20mg od, with DLTs observed at doses above 20mg od. PK data indicate exposures in the preclinical efficacy range. Metabolomic changes in urinary lactate and ketones correlate with on-target activity. A patient with undiagnosed tumour-associated lactic acidosis experienced a DLT with exacerbation of this following a single 10mg dose of AZD3965, again indicating target engagement.

Conclusion: The MCT1 inhibitor AZD3965 can be administered to patients at doses which engage the drug target, with a RP2D of 10mg bd po. Observed DLTs were primarily dose dependent, asymptomatic, reversible changes in retinal function, an expected on-target effect. Part 2 of the study has just completed recruitment of patients with diffuse large B cell lymphoma, a tumour that predominantly expresses MCT1.

Tea Pemovska

EDUCATION

PhD in cancer chemical systems biology/personalized cancer medicine, Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland
MSc in Pharmaceutical Sciences and Drug Innovation, Utrecht University, Utrecht, The Netherlands
BSc in Biomedical Sciences, University College Utrecht, Utrecht, The Netherlands

WORK CAREER

Position

Postdoctoral fellow

Affiliation

CeMM, Research Center for Molecular Medicine of the Austrian Academy of Sciences

Representative Awards

EMBO long term fellowship, Best doctoral thesis for 2015 of the Faculty of Medicine, University of Helsinki, Young Scientist Special Prize, Biomedicum Helsinki Foundation, 3 Best of Free Contributions Award, Acute Leukemias XV, Munich, Germany

Research Areas of Interest

translational cancer research, chemical systems biology, cancer metabolism, hematological malignancies

ABSTRACT

Identification and targeting of metabolic vulnerabilities relevant to myeloid leukemias

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Introduction: Cellular metabolism represents a dynamic network of regulated pathways that is frequently reprogrammed in the context of cancer. Interrogation of this network with high-throughput screening approaches can unravel context biology as well as identify cancer-specific metabolic vulnerabilities.

Experimental: To systematically study the consequences of metabolic perturbation, we assembled a metabolic drug library covering 243 compounds and phenotypically characterized it in a panel of 15 myeloid leukemia cell lines. Each drug was tested in 5 different concentrations covering 10 000 concentration range, facilitating the generation of dose response curves and area under the curve scoring.

Results: Analysis of the drug response profiles revealed that roughly 30% of the tested compounds affected cell viability with the top effective compounds targeting nucleic acid synthesis, oxidative stress, and the PI3K/mTOR pathway. Unsupervized hierarchical clustering of the drug sensitivity profiles stratified the cell lines in 5 functional taxonomic groups, with the response pattern of 19 compounds significantly impacting the grouping. Selective sensitivities were detected to the lactate transporter inhibitor AZD3965, the PI3K inhibitor pictilisib, and the fatty acid synthase inhibitor GSK2194069, which could be explaining by varied gene expression and target/process dependency.

Conclusion: Our data highlights that the metabolic drug library is a valuable tool to probe cellular metabolism and pinpoints that metabolic perturbation may hold promise as a therapeutic strategy for myeloid leukemias and beyond.

Valerio Pazienza

EDUCATION

2017 Ph.D. in "Health Food Innovation and Management" University of Foggia (Italy)
2005 Specialization School in Clinical Biochemistry University of Camerino-MC (Italy)
2002 Master in Biotechnology University of Urbino (PU) Italy
2001 Licensed Professional Biologist University of Urbino (PU) Italy
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WORK CAREER

Position
Principal Investigator

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Representative Positions
2015 / March - April Visiting Scientist at Tallinn University of Technology, TUT (Estonia) Department of Gene therapy. Research Field: microbiota studies in cancer xenograft animal model.
2014 / September - October Visiting scientist at "Chromosome Engineering Research Center" University of Tottori (Japan) and at "Laboratory of Animal Research Center" Institute of Medical Science, University of Tokyo (Japan)
2013 / October Visiting scientist at "Chromosome Engineering Research Center" University of Tottori (Japan). Research Field: Metabolism and liver cancer in MacroH2a transgenic mouse.

Representative Awards
- Swiss Society of Gastroenterology 2006 for establishing a new in vitro model of different hepatitis C virus genotypes
- Young Research Award (GR-2010-2311017-148) funded by the Italian Ministry of Health.
- 1/8/2016 the Presidency of the Republic awarded Dr. Pazienza Valerio for the merit of public health (https://www.quirinale.it/onorificenze/insigniti/339804).

Research Areas of Interest
microbiota in non communicable diseases

ABSTRACT

Pharmacomicrobiomics: exploiting the diet-drug-microbiota interactions in anticancer therapies

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Despite continuous advances in cancer related therapies, resistance to standard drugs and adverse effects still represent an important cause of therapeutic failure.

Cancer is a major health burden worldwide being among the top two killing disease in the frame of non comunicable diseases that are responsible of the 70% of deaths worldwide. There is a growing evidence that gut bacteria can affect the response to chemo- and immunotherapeutic drugs by modulating either efficacy or toxicity. Moreover, intratumor bacteria have been shown to modulate chemotherapy response. At the same time, anticancer treatments themselves significantly affect the microbiota composition, thus disrupting homeostasis and exacerbating discomfort to the patient. In this lecture is presented the existing knowledge concerning the role of the microbiota in mediating chemo and immunotherapy efficacy and toxicity and the ability of these therapeutic options to trigger dysbiotic condition contributing to the severity of side effects. In addition, we discuss the use of probiotics, prebiotics, synbiotics, postbiotics, and antibiotics as emerging strategies for manipulating the microbiota in order to improve therapeutic outcome or at least ensure patients a better quality of life all along of anticancer treatments.

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Oral Communitations
Abstracts

04

OC1 Surviving metabolism: acidity as a selection pressure in colorectal cancer cell lines

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Introduction: Extracellular acidity is a chemical signature of tumours. It arises because the raised metabolic rate in cancer cells releases large quantities of lactic acid and CO2 into the tumour microenvironment[1,2]. Dysregulated pH has been shown to perturb or even kill cancer cells. Although targeting acidity is a good candidate for the therapeutic management of tumour growth[3], so far, none of the major approved therapies are based explicitly on disrupting acid handling and/or signalling. Using a large panel of colorectal cancer (CRC) cell lines, our aim was to identify the molecular processes that provide a survival benefit to cancer cells living under acid-stress.

Methods: We investigated pH-related physiology in sixty-eight CRC cell lines by measuring (i) survival over time by a sulphorhodamine B (SRB) based assay, (ii) time course of extracellular acidification as a readout of metabolic output, and (iii) intracellular pH (pHi) by fluorescence imaging. Measurements were performed on an imaging plate reader (Cytation 5, Biotek) and repeated over a range of extracellular pH (pHe) levels.

Results: CRC cell lines displayed dramatic differences in their sensitivity to pHe. A 50% reduction in survival was associated with pHe values of 7.38 (most sensitive: Iscerol) to below 6.8 (least sensitive: SW1222). Furthermore, the cell lines differed in their ability to regulate pHi in response to changes in ambient pHe, quantified in terms of the slope of their pHi-pHe relationship. This metric of pHi-control ranged from 0.18 (Colo678) to 0.58 (Iscerol), representing strong to weak pHi control, respectively. Based on their survival curves and culmulative acid production rates, CRC cell lines were grouped into 'acid sensitive' or 'acid resistant', and 'low metabolic acid production' or 'high metabolic acid production'.

Conclusions: We established a database that provides a comprehensive appraisal of the pH phenotype for individual CRC cell lines. This information allows us to correlate phenotype with mutations and gene expression profiles of these cell lines, and to identify molecular processes involved in acid handling and signalling.

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OC2 Chronic acid adaption of cancer cells profoundly alters cellular pH homeostasis, lipid metabolism and lysosomal biogenesis pathways

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Introduction: The acidic tumor microenvironment profoundly impacts cancer cell behavior, favoring an aggressive, invasive phenotype[1,2]. The mechanisms involved remain incompletely understood and have generally only been described for individual cell types and selected phenotypes. The aim of this work is to provide a comprehensive understanding of acid-induced changes in cancer cells.

Experimental: Panc1 (pancreatic), MCF7, and MDA-MB-231 (luminal A and triple-negative breast) cancer cells were cultured at pH 6.5 or 7.6, respectively, for 1-3 months. Protein expression, pH homeostasis, and lipid metabolism were analysed by western blotting, immunofluorescence microscopy, real-time intracellular pH (pHi) imaging, BrdU, shotgun lipidomics, and motility- and 3D growth assays.

Results: Relative to cells cultured at pH 7.6, acid-adapted lines exhibited increased steady state pHi at pH 7.4 and upregulation of the Na+-HCO3- cotransporter NBCn1, whereas expression of Na+/H+ exchanger NHE1 and lactate-H+ cotransporter MCT4 was generally unchanged. Acid-adapted cells exhibited increased lipid droplet formation, a shift toward lipids with polyunsaturated fatty acids, decreased expression of acyl-CoA:cholesterol acyl transferase-1 (ACAT1), and significant cell-type specific changes in cholesterol- and sphingomyelin levels. Acid-adapted cells exhibited more peripherally localized lysosomes and increased expression of lysosomal membrane proteins LAMP1 and -2 and transcription factor EB (TFEB), a master regulator of lysosome biogenesis. Proliferation and 3D growth were modestly decreased, and migration increased, in acid-adapted cells.

Conclusions: Chronic acid adaption of cancer cells is associated with profound changes in pHi homeostasis, lipid metabolism, and lysosome biogenesis, some of which are similar, while others differ greatly between different cancer types. Ongoing work investigates the links between these pathways and their precise roles in tumor progression.

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Acknowledgements: We are grateful to Katrine F. Mark for excellent scientific assistance.

OC3 Acetyl-CoA metabolism supports multi-step pancreatic carcinogenesis

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Introduction: Mutant KRAS is thought to initiate pancreatic tumorigenesis, orchestrating a program that leads to cell de-differentiation, proliferation, and symbiotic cooperation with neighboring cells, enabling the cancer cells to thrive in a particularly harsh microenvironment. Recent studies have highlighted the role of metabolites in regulating the epigenome. Although oncogenic KRAS is known to reprogram cellular metabolism, the role of metabolic control of the epigenome in pancreatic tumorigenesis is poorly understood.

Experimental: We showed that expression of KRASG12D in mouse pancreas promotes elevated histone acetylation levels in pancreatic acinar cells, and that this precedes tumor development. We hypothesized that augmented acetyl-CoA metabolism may play a role in facilitating pancreatic tumorigenesis. To test this, we generated mice deficient for *Acly* (acetyl-CoA producing enzyme) in pancreas (*Pdx1-Cre; Aclyf/f* mice).

Results: In the context of KRASG12D expression, ACLY deficiency reduces histone acetylation levels in pancreatic acinar cells and impairs formation of neoplastic lesions. ACLY deficiency also impairs pancreatitis-induced tumor development. In testing roles for acetyl-CoA-dependent processes in ADM, we found that targeting either histone acetylation by BET inhibition or cholesterol synthesis with statins suppressed tumor onset.

Conclusions: The findings indicate that ACLY-dependent metabolic and epigenetic remodeling promote tumor development and point to the potential to target acetyl-CoA metabolism for pancreatic cancer. Current efforts to elucidate the metabolic and epigenetic roles of ACLY in the context of pancreatic tumorigenesis will be presented.

References:

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Acknowledgements: This work was supported by the 2014 Pancreatic Cancer Action Network–AACR Career Development Award, grant number 14-20-25-WELL, and the NIH grants R01CA174761 and R01CA228339 to K.E. Wellen. The work is currently supported by the iCARE-2 fellowship (Project code: 22550) from AIRC (Associazione Italiana Ricerca sul Cancro) to A. Carrer.

OC4 Regulation of energy metabolism by formate

Kristell Oizel[1], Jorge Fernandez-de-Cossio-Diaz[2], Johannes Meiser[3], Jacqueline Tait-Mulder[1], Sergio Lilla[1], Giovanny Rodriguez Blanco[1], David Sumpton[1], Gillian M Mackey[1], Sara Zanivan[1,4] and Alexei Vazquez[1,4]

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Introduction: Formate supplies one-carbon units for the biosynthesis of RNA and DNA in proliferating cells. Yet, less attention has been paid to the interaction between the formate dependent synthesis of adenine nucleotides and energy metabolism.

Experimental: Here we use a combination of theoretical simulations and in vitro genetic modulation to investigate the link between formate and energy metabolism.

Results: We uncover that endogenous formate production induces the simultaneous activity of glycolysis and oxidative phosphorylation while repressing AMPK activity. In the absence of endogenous formate production, exogenous formate can recapitulate the effect. In vivo data for mouse and human cancers confirms the association between increased formate production and energy metabolism.

Conclusions: We conclude that endogenous or exogenous formate induces glycolysis and mitochondrial activity, providing a biochemical mechanism for the Warburg effect that does not require mitochondrial repression.

Acknowledgements: This work was supported by Cancer Research UK C596/A21140 and the Cancer Research UK Glasgow Centre (C596/A18076). This project has received funding from the European Unions Horizon 2020 research and innovation programme MSCA-RISE-2016 under grant agreement No. 734439 INFERNET. JM was supported by a DFG Fellowship (Grant Number ME 4636/2-1) and by a FNR ATTRACT fellowship (Grant Number: A18/BM/11809970).

OC5 PI3K-C2γ Loss Promotes Pancreatic Cancer through mTOR Regulation and Metabolic Rewiring

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Introduction: Pancreatic Ductal Adenocarcinoma (PDAC) is the fourth leading cause of cancer death. The identification of critical biochemical mechanisms underlying the pathogenesis of PDAC is essential to design alternative therapeutic approaches for this disease. In PDAC, enhanced activation of mTOR pathway is required for tumor growth and metabolic rewiring1, but how mTORC1 signaling is regulated is unclear. PI(3,4)P2, produced by class II PI3K as PI3K-C2γ2, is a negative regulator of mTORC13. PI3K-C2γ is mainly expressed in pancreas and it is lost in 6% of pancreatic cancer patients, suggesting a tumor suppressor function in PDAC and in mTOR-dependent metabolic adaptation.

Experimental: Mouse model of PDAC (K-RASG12D/Trp53R172H/CrePdx1) was crossed with mouse strain lacking PI3K-C2γ expression. Mice were weekly followed for survival, tumor appearance and growth. Tumor lesions were evaluated by histopathological and immunofluorescence analysis. Functional in vitro and in vivo experiments were performed.

Results: Given that low PI3K-C2γ expression is significantly associated with poor survival, we modeled PI3K-C2γ loss in a mouse model of PDAC (KPC). Loss of PI3K-C2γ in KPC mice reduces mice mean survival rate (18 vs 36 weeks) and drives rapid progression to PDAC. In addition, PI3K-C2γ loss promotes the metabolic rewiring of PDAC, through the upregulation of mTORC1 pathway. At the lysosomal level, PI3K-C2γ sensitizes cells to glutamine but not glucose deprivation. Taken together, these results consolidate PI3K-C2γ as a new candidate in mTOR-dependent PDAC progression, impacting on tumor metabolism.

Conclusions: These findings establish PI3K-C2γ as a PDAC tumor suppressor and suggest that the metabolic phenotype of PI3K-C2γ-deficient tumors can be exploited by specific therapeutic strategies.

References:

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Acknowledgements: MCDS., FG. and JPM are supported by AIRC/FIRC fellowship.

OC6 Metabolic intratumoural heterogeneity in cancer

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The small intestine is a rapid renewing epithelium formed by different cell types. At the bottom of the intestinal crypt, stem cells divide every 24 hours to maintain the intestinal homeostasis. Stem cells reside between terminally differentiated Paneth cells (PCs) that support stem cell function by secreting growth factors (Egf, Wnt and Notch ligands). We have recently shown that cellular metabolism plays an important role in the stem cell niche[1]. In the intestinal crypt, PCs support stem cell function by secreting lactate which is consumed by mitochondria in stem cells. Currently, we study how this "metabolic teamwork" takes place in colorectal tumours. In particular, we investigate the contribution of glucose metabolism to stem cell function and identity. We use organoid technology to culture healthy colon and tumours from patients. In these organoids we have introduced an Lgr5 based mini gene to trace cancer stem cells[2]. Our research shows that there is metabolic and proliferative heterogeneity within tumour organoids. Interestingly, we found that the way that cells metabolize glucose is strongly linked with their identity and tumorigenic potential. Importantly, our observations are consistent across a large variety of genetic backgrounds, indicating that these are conserved mechanisms that lead to successful tumour establishment and growth. Our research contributes to a better understanding of the mechanisms of cancer cell plasticity and tumor heterogeneity.

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OC7 Characterization of the metabolic control of brain metastasis in breast cancer

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Online publication not authorized.

OC8 Loss of ISG15 expression and ISGylation Reduces Mitophagy and the Functionality and Metabolic Plasticity of Pancreatic Cancer Stem Cells

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Introduction: Pancreatic cancer stem cells (PaCSCs) drive pancreatic cancer tumorigenesis, chemoresistance and metastasis. While eliminating this subpopulation of cells would theoretically result in tumor eradication, PaCSCs are extremely plastic and can successfully adapt to targeted therapies, such as those that target mitochondrial respiration.

Experimental: Using RNAseq to transcriptionally distinguish CSCs from non-CSCs, we observed that PaCSCs of different primary pancreatic ductal adenocarcinoma tumors exhibit increased transcriptional activation of genes that participate in the ubiquitin (Ub) conjugation pathway. We next investigated whether alternate Ub-like pathways were also enriched in CSCs and discovered that PaCSCs not only upregulate the expression of the interferon stimulated gene 15 (ISG15), but PaCSCs exhibit increased protein ISGylation compared to non-CSCs. We therefore eliminated ISG15 expression via CRISPR-mediated genomic editing to evaluate the effect of ISG15 loss on the biology and metabolism of PaCSCs.

Results: GSEA analysis revealed that ISG15-high tumors and cells are enriched in genes associated with oxidative phosphorylation (OXPHOS). CRISPR-mediated ISG15 genomic editing not only reduced overall ISGylation, impairing PaCSCs self-renewal and their in vivo tumorigenic capacity, but ISG15 loss resulted in decreased mitochondrial ISGylation concomitant with increased accumulation of dysfunctional mitochondria, reduced OXPHOS and impaired mitophagy. Importantly, disruption in mitochondrial metabolism affected PaCSC metabolic plasticity, making them susceptible to prolonged inhibition with metformin in vivo.

Conclusions: ISGylation is critical for optimal and efficient OXPHOS by ensuring the recycling of dysfunctional mitochondria, and when absent, a dysregulation in mitophagy occurs that negatively impacts PaCSC stemness and their metabolic plasticity, making them highly susceptible to metabolic inhibitors such as metformin.

OC9 P53 sustains oxidative metabolism and delays the energetic collapse caused by targeting respiratory complex I in combination with low glucose

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4. Istituto di Biomembrane, Bioenergetica e biotecnologie molecolari - IBIOM - CNR.

Introduction: Respiratory complex I (CI) has been recognized as one of the targets of metformin, and its inhibition decreases cancer cells growth, albeit this is not sufficient to eradicate disease. Recently, a combination of metformin and low glucose was shown to be lethal for tumors1, although it is likely that different drivers of tumorigenesis may lead to different degrees of efficacy of such combo.

Experimental: To induce a metabolic collapse we grew different cancer cell models in low glucose (1mM). The analyzed models were either CI-deficient cells or CI-competent treated with specific CI inhibitors or metformin2,3. Further models were generated to be isogenic except for TP53, whereby obtaining cells devoid of CI with either wild-type p53, or p53-null, or expressing two different p53 pathogenic mutations.

Results: The combination of CI-deficiency and low glucose was lethal for all the CI-deficient cell models (colon and thyroid carcinoma and osteosarcoma). Nonetheless, the p53 genetic status played a relevant role in delaying cell growth arrest and death. We observed that expression of mutant, but not of wild-type p53, was completely abolished after 24h of growth in low glucose, only in CI-deficient cells. This feature was not due to decreased mRNA expression or to an increased proteasomal degradation, but to a general protein synthesis arrest triggered by the combo, which was much slower to occur in the presence of a wild-type p53. Indeed, the protein levels of the latter decreased but persisted, and were sufficient to trigger SCO2 expression, likely compensating the energetic impairment.

Conclusions: p53 mutated tumors are unable to compensate the metabolic change following CI inhibition and low glucose, and may be more sensitive to this combined therapeutic approach.

References:

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2. Porcelli AM et al, Hum Mol Genet 2010;
3. Kurelac I et al, Nat Commun 2019.

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OC10 Role of autophagy in the interactions between adipocytes and breast cancer cells

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Online publication not authorized.

OC11 Obesity and Triple Negative Breast Cancer: is apelin a new key target?

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OC12 Polyunsaturated fatty acids reduces in vitro tumor growth of colorectal cancer patient-derived organoids

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Introduction: Epidemiological data support that a high-fat intake typical of Western diet, increases the risk of colon cancer (CRC). It has been demonstrated that, high fat diet (HFD) and exposure to saturated fatty acids - such as palmitic acid (PA) - augments intestinal progenitors and their tumorigenicity[1]. Instead, polyunsaturated fatty acids, such as docosahexaenoic acid (DHA) are shown to be protective against CRC; however experimental evidence supporting this anticancer effect, as well as the mechanism, is still lacking[2]. We investigated the potential anti-CRC activity of polyunsaturated fatty acids in our collections of patient-derived CRC organoids.

Experimental: Cell viability was determined by ATP releases (Cell Titer Glo®) and cell proliferation by ki-67 or EdU. Lgr5 or SOX2 RNA levels were determined by qPCR. To assess the colony-forming efficiency we dissociated CRC-organoids to single cells and quantified the number of growing organoids.

Results: DHA decreased CRC growth in vitro, while PA did not show any effect respect to control. Tumor growth inhibition was mainly caused by cell proliferation rate reduction. Notably, this effect was more prominent in KRAS-mutated compared to KRAS-wild type tumors. We further analyzed the effect of DHA or PA on organoids tumor initiating cells by assessing stem cell markers and clonogenic capacity. DHA treatment diminished Lgr5 and SOX2 RNA levels in KRAS-mutated organoids while PA treatment did not modify expression levels. Besides, DHA treatment decreased colony formation respect to control while PA did not. Even DHA-pretreated organoids showed decreased colony efficiency compared to those that were not pre-treated, supporting a role for DHA in modulating the stem cell number in organoids.

Conclusions: Our results indicate that polyunsaturated fatty acids affect organoid-initiating capacity and tumor growth supporting a potential role for prevention and treatment of CRC.

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OC13 Epigenetic-Metabolic interplay in renal cell carcinoma: role of lactate on sirtuin's modulation

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Introduction: Renal cell carcinomas (RCC) are the most lethal of the common urological cancers. Recently, metabolic reprograming and epigenetic deregulation were recognized as cancer hallmarks and their interactions have been associated with cancer aggressiveness. Lactate has been recently shown to modulate epigenetic mechanisms, leading to histone deacetylases inhibition[1]. Herein, we investigated the interaction between metabolism and epigenetics in RCC.

Experimental: The effects of lactate, nicotinamide (NAM) and alpha-cyano-4-hydroxycinnamate (CHC) on epigenetic enzymes and cell phenotype were evaluated in normal kidney and RCC cell lines. Additionally, in vivo effect was also assessed by CAM model assay. Finally, HIF-1a, SIRT1 and SIRT6 immunoexpression was evaluated in human RCC and normal renal tissues.

Results: Lactate inhibited SIRT1 and SIRT6 in RCC cells and normal kidney cells, increasing H3K9 and H4K16 acetylation. Cells exposed to lactate displayed pronounced glycolytic phenotype, disclosing increased cell migration and invasion. NAM treatment paralleled lactate effects, promoting cell aggressiveness, whereas CHC reversed them. Lactate and NAM exposure increased tumors' size, while CHC associated with diminished tumors growth in vivo CAM model. Primary RCC disclosed HIF-1a upregulation, whereas SIRT1 and SIRT6 expression was downregulated comparing with normal tissues.

Conclusions: In RCC, lactate enhances tumor cell aggressiveness and modulates normal cell phenotype inducing malignant-like features, through SIRTs downregulation. Therefore, tumor metabolism might be a promising therapeutic target in RCC.

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OC14 Genetic disruption of the cystine importer xCT (SLC7A11) reduces growth, survival and tumorigenicity and increases sensitivity to chemotherapy of pdac cells (Capan-2 and MiaPaCa-2)

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The system xCT acts as a Na+-independent and Cl--dependent antiporter of the anionic forms of cystine (oxidized form of cysteine) and glutamate. The activity of this system is crucial for the maintenance of the cellular redox balance as the uptake of cystine is required for the synthesis of intracellular glutathione (GSH). GSH represents the major antioxidant molecule of the cell, also involved in biotransformation of xenobiotics and essential in protein folding. Recent pharmacological studies suggested xCT as potential target for redox-based anti-cancer therapy. In order to further investigate this issue, particularly in pancreatic ductal adenocarcinoma (PDAC) we made a knockout of the xCT transporting subunit in Capan-2 and MiaPaCa-2 cell lines using the CRISPR-Cas9 technique. These cells were then characterized for GSH content, proliferation, clonogenicity, and survival. Considering that cysteine-to-cystine in blood is subjected to changes, focus of the study was also placed on the importance of the xCT for these cells to make tumors in vivo.

Both xCT-KO cell lines had markedly reduced GSH content, leading to an increased accumulation of oxidative stress marker - lipid peroxides, which ultimately resulted in massive cell death by ferroptosis. As expected, cultivating xCT-KO cells under reducing conditions (N-acetyl-cysteine or β-mercaptoethanol) that allow bypassing the activity of xCT, restored cellular GSH content, proliferation and survival of xCT-KO cells. Also, accumulation of lipid peroxides and survival of the mutant cells were prevented by two inhibitors of ferroptosis: Vitamine E or an iron chelator - deferoxamine (DFO). Present study showed that pharmacological inhibition of xCT by a low concentration of erastin (1 µM) phenocopies its genetic disruption, and increases susceptibility of PDAC cells to chemotherapeutics such as gemcitabine or cisplatine.

Furthermore this study showed for the first time that genetic disruption of xCT negatively affects tumorigenic potential of both cell lines. However, the cells were still able to slowly develop tumours in vivo suggesting two possibilities: 1) residence mechanisms are involved in adaption xCT-KO cells to in vivo environment and/or 2) cysteine-to-cystine balance in the tumoral microenvironment permits adaptation and survival of xCT-KO in mice by potentiating cysteine transport.Accordingly, the cystine importer xCT appears to be a promising therapeutic target for PDAC treatment development as its inhibition/disruption disturbs significantly the redox equilibrium, proliferation, survival and chemoresistance of PDAC cancer cells.

OC15 Inhibition of glutamine synthetase by glufosinate ammonium skews macrophages towards a M1-like phenotype and inhibits tumor metastasis

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TAMs are known to actively participate to the metastatic process by contributing to different steps in the metastatic cascade[1], such as angiogenesis and cancer cell invasion[2,3,4]. Recently metabolism has been highlighted as important mediator of macrophage function through the discovery of the mechanisms that, behind these metabolic changes, strongly impact on immune function[5], potentially modulating cancer development and metastasis formation. We have demonstrated that genetic targeting of macrophagic glutamine synthetase (GS), that generates glutamine from glutamate, impairs the immunosuppressive, proangiogenetic and prometastatic function of M2-like macrophages[6], highlighting the opportunity to target this enzyme in the treatment of cancer metastasis.
In this study we test the effects of the GS inhibitor glufosinate ammonium on macrophage function both ***in vitro*** and ***in vivo***. Glufosinate treatment skews IL-10 stimulated macrophages towards a M1-like phenotype. In tumor bearing mice glufosinate repolarizes TAMs towards a M1-like state, leading to metastasis inhibition with no signs of liver or brain toxicity. These results demonstrate the importance of metabolic immunotherapeutic strategies in promoting the revert of TAMs function against cancer metastasis.

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OC16 New target molecules depict T regulatory cells in Malignant Mesothelioma

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Poster
Abstracts

05

P1 Characterization of glycolytic metabolism of oesophageal carcinomas and evaluation of its potential as therapeutic target

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Introduction: Malignant cells exhibit a preference for glycolytic metabolism for energy production, even in the presence of oxygen, a phenomenon denominated "Warburg effect". Cancer cells have high glycolytic rates leading to an increase in lactate produced, which is transported to the tumour microenvironment via monocarboxylate transporters (MCTs), thus contributing to increased proliferation, migration and survival of tumour cells. The role of MCTs is poorly understood in oesophageal carcinomas. The role of MCTs is poorly understood in oesophageal carcinomas. To achieve our objectives, we performed a detailed characterization of the expression of MCTs and other metabolic markers in a clinical series of two histological types of esophageal cancer, oesophageal squamous cell carcinoma (OSCC) and oesophageal adenocarcinoma (OAC). Expression of MCTs was subsequently correlated with CD147 and with clinical data. Then a metabolic characterization of squamous cell carcinoma cell lines of the esophagus was performed and the effect of inhibition of MCTs in these cell lines was evaluated by levels of biomass and cellular metabolism.

Experimental: In vitro studies have shown that the dual silencing of MCT1 and MCT4 partially corroborated this assumption since demonstrated a decrease of cancer cell viability but, paradoxically, with little effect on glucose consumption and extracellular lactate delivery. 1. Characterization of the immunohistochemical expression of MCTs, CD147 and metabolic markers. The analysis of metabolic markers expression will be performed in a series of oesophageal carcinomas. Analysis of MCTs, CD147 and metabolic markers such as GLUT-1, CAIX, LDH-A, PDK, HKII, HIF-1α, expressions in oesophageal carcinomas samples will be performed by immunohistochemistry, using specific antibodies. 2.
Evaluation of the effects of MCT inhibition in tumour cell lines of oesophageal carcinomas. The upregulation of glycolysis in cancer cells is harmless to themselves but toxic to the surrounding cells, this adaptation was achieved with the help of MCTs that malignant cells have. Therefore, characterization of the expression of MCTs, CD147 and others metabolic markers (GLUT-1, HKII, LHD-A, CAIX, HIF-1α and PDK) were assessed in different oesophageal carcinoma lines, by immunocytochemistry and Western blot. In order to understand the role of MCTs in oesophageal carcinomas, we evaluated the effect of MCT activity inhibition (MCT inhibitors) on tumour cell viability, proliferation, migration, apoptosis and metabolism in different oesophageal AC and SCC cell lines. For cell viability and cell proliferation the sulforhodamine B and BrdU cell proliferation assay were assessed, respectively. Apoptosis was evaluated by Annexin V/PI and cell migration will be assessed by the wound healing assay. Due to the enhanced aerobic glycolytic metabolism, cancer cells consume great amounts of glucose and produce large amounts of lactate, and because of that glucose consumption, lactate production and extracellular pH will demonstrate the role of MCTs.

Results: This study showed that tumour location and age are important factors for low survival in adenocarcinoma. Gender is a factor of low survival for esophageal carcinoma. It also showed that the expression of MCT1 and MCT4 in the plasma membrane is more evident in squamous cell carcinoma than in adenocarcinoma and that for squamous cell carcinoma, MCT4 and CA IX can be considered prognostic factors for low survival. In vitro studies have shown that the dual silencing of MCT1 and MCT4 leads to a decrease in cell viability but had little effect on glucose consumption and lactate production. However, further studies will be needed to observe what this dual inhibition may cause in proliferation and migration. Only in this way will it be possible to know if MCTs could be rational therapeutic targets for use in this type of cancer. The results obtained in this study intended to contribute to a better understanding of role of MCTs, and to open new therapeutic possibilities for esophageal cancer.

Conclusions: This study indicated that MCT4 and CA IX high expressions can be considered as prognostic factors for patients' low survival.

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P2 The metabolic reprogramming of tumors cells under acidosis enhances the anti-proliferative and metabolic activity of dichloroacetate, a PDK inhibitor

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Introduction: Many cancer cells present an exacerbated glycolytic flux that provides advantage for growth. By inhibiting pyruvate dehydrogenase kinase (PDK), dichloroacetate (DCA) shifts the metabolism from glycolysis to mitochondrial oxidation and decreases the proliferation of various cancers cells1. While extracellular acidosis is a common feature observed in glycolytic tumors2, acidosis has never been considered as a potential modulator of DCA activity. Here, we examined whether acidic pH could influence the effects of DCA on the bioenergetics and growth of cancer cells.

Experimental: We used a long-term selection of cancer cells able to proliferate under acidic conditions and various techniques including 13C-NMR spectrometry to measure DCA uptake and evaluate metabolic fluxes.

Results: We found that DCA exerts more profound growth inhibitory effects in acid-adapted clones than in parental cancer cells, together with a higher uptake of DCA, a lower PDK abundance and a higher decrease in PDH phosphorylation (the PDK target). Moreover, daily DCA administration to mice led to a significant delay in tumor growth from acid-adapted cells but not from parental cells. A double metabolic shift was further observed in response to DCA with a reduction in lactate release and an increase in glutamine uptake. The lactate reduction was higher in acidic clones. We observed that DCA reduced pentose phosphate pathway activity and increased mitochondrial apoptosis. These effects were more pronounced in acid-adapted cells and confirm that metabolic shifts induced by DCA account for its anti-proliferative action. Finally, the combination of DCA with a glutaminase inhibitor significantly enhanced the growth inhibitory effect of DCA.

Conclusions: Overall, the interplay between acidosis and DCA exposure leads to metabolic reprogramming that increases DCA activity.

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P3 Low pH directly boosts osteosarcoma aggressiveness by reducing intercellular adhesion and enhancing intravasation

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Introduction: Osteosarcoma (OS) is the most common primary malignant bone tumour with a high tendency to form lung metastasis. We previously demonstrated that the OS microenvironment is acid.1 The low pH stimulates a secretome from tumour-associated mesenchymal stromal cells (MSC) with pro-migratory effects on OS cells.1 However, low pH might have an additional role in OS metastasis. In this study, by using 3D static and dynamic models, we investigated on the activity of low pH on directly inhibiting the formation of intercellular junctions between OS cells, and on OS cell motility and intravasation.

Experimental: OS cells were cultured alone or mixed with MSC in 3D spheroids at pH 7.4 or 6.7. Spheroids were used for 1) immunostaining of Claudin-8, ICAM1, tubulin and Connexin-43; 2) transmission electron microscopy (TEM) analysis; 3) western blotting of ICAM-1 and Claudin-8; 4) seeding in a microfluidic device to assay OS cell migration, endothelial cell fenestration, and OS intravasation. The microfluidic device included a channel with 3D OS/MSC cultures that were perfused by an adjacent channel that included a vessel-like structure with HUVEC cells.

Results: Incubation for 5 days with extracellular acidosis completely impaired the expression of proteins needed for junction formation in OS cells. The reduction of cell-to-cell adhesion structures was also confirmed by TEM. In agreement with a less attachment-dependent phenotype, in the microfluidic device, the low extracellular pH enhanced the migration and intravasation of OS cells.

Conclusions: Extracellular acidosis is directly responsible for the metastasis formation of OS through the inhibition of cell-to-cell adhesion, and the direct stimulation of tumour cell migration and intravasation. Our data unravel novel mechanisms for the OS metastasis that should be considered for the development of novel treatments to prevent OS recurrence.

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P4 Characterization of the molecular and functional consequences of MCT1 inhibition in cancer treatment

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P5 Pancreatic stellate cell activation is coordinated by environmental pH and mechanical stress

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Introduction: Pancreatic stellate cells (PSCs) are stromal cells of the pancreas that become activated in various diseases, prominently in pancreatic cancer. Pancreatic cancer is coupled to changes in the extracellular environment of the stroma, including mechanical stiffness and interstitial pH. Tumorous transformation of the pancreatic ductal cells impairs ductal HCO3- secretion, which in turn leads to a relative alkalinization of the interstitial pH of the pancreas1. In this study we aim to model and elucidate how changes in extracellular pH (pHe) and mechanical stress affect the intracellular pH (pHi) homeostasis and activation of pancreatic stellate cells.

Experimental: Following isolation, we cultured murine PSCs for up to 120 hours in media buffered to different pHe on a stiff substrate or on polyacrylamide gels of physiological stiffness. Cellular viability, activation and proliferation were assessed using immunocytochemistry and flow cytometry. To investigate the functional expression of potential pH-dependent sensors, regulators and transporters of PSCs, we performed RT-qPCR, Western blot and pHi measurements.

Results: Alkaline pHe substantially activates PSC as evidenced by an increase in cell size and α-SMA positivity, especially on a rigid substrate. Similarly, cell proliferation, cell cycle progression and nuclear Yap localization are also facilitated in an alkaline pHe environment. Multiple pH regulators are highly expressed in activated PSCs cultured in pHe 7.4 compared to freshly isolated cells and cells cultured in pHe 6.6. Also, NHE1 activity is enhanced in activated PSCs at pHe 7.4 as compared to inactive PSCs at pHe 6.6.

Conclusions: In summary, extracellular acidification keeps PSCs in a quiescent state, whereas upon pHe alkalinization PSCs enter the cell cycle on stiff substrates through Yap/Taz signaling.

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P6 The role of adipocyte G0/G1 Switch Gene 2 (G0S2) in the crosstalk between cancer cells and adipocytes

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Among the stromal cells present in the tumor microenvironment, adipocytes were reported to up-regulate cancer cells migration and invasion. The effects of fatty acids released from adipocytes on the phenotype of tumor cells were also recently reported in vitro. On the other hand, tumor cells were reported to alter adipocyte phenotype notably by increasing lipolysis. However, the molecular mechanisms triggered in adipocytes by tumor cells and the precise contributions of adipocyte lipolysis in tumor phenotype in vivo are still poorly understood.

In this study, we search to identify key adipocyte genes involved in the crosstalk between tumor cells and adipocytes. For this purpose, we evaluated the consequences on adipocytes (differentiated 3T3-L1) of co-culture with prostate cancer cells.

We observed that among the 3 genes regulating the first and rate-limiting step of triacylglycerol hydrolysis (the adipose triglyceride lipase (ATGL), its coactivator CGI-58 and its inhibitor G0S2 which locks the system and prevents triacylglycerol hydrolysis), G0S2 is by far the most strongly repressed by the presence of tumor cells. This regulation is partly mediated by the inhibition of glycolysis due to the acidosis induced by the metabolism of cancer cells. Lipophagy was not involved in the regulation of lipolysis in our model while G0S2 downregulation was associated with an enhanced lipolysis and the re-expression of G0S2 in adipocytes repressed lipolysis.

Our results support a key role for adipocyte G0S2 in the crosstalk between adipocytes and cancer cells. The analyses of the effects on tumor growth and metastasis of a specific overexpression of G0S2 in adipocytes and the characterization of the intracellular signaling pathways involved in the regulation of G0S2 are ongoing.

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P7 Akt-dependent regulation of NHE1 and its potential role in driving cancer growth

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Introduction: Increased cytosolic pH (pHc) is a hallmark for cell division and tumorigenesis. Na+-H+ exchangers (NHEs) extrude cytosolic H+ into extracellular space and thus regulate pHc in human cells. NHEs are required for growth factor-induced pHc increase and contribute to the regulation of cell cycle progression1,2. NHE1 can be phosphorylated by Akt and Erk1/2, which may play an important role in regulation of NHE13. However, the exact function of Erk1/2 and Akt-dependent phosphorylation on NHE1 regulation is not fully known.

Experimental: Impact of NHE1 on cell cycle progression was studied by flow cytometry in normal epithelial RPE cells treated with a NHE1 inhibitor (NHEi) and the impact of NHE1, Akt, and Mek inhibition on pHc was measured by ratiometric microscopy of cells expressing a pH-sensitive GFP (pHluorin). Cell growth/proliferation was assessed by MTT assay using A431 carcinoma cells expressing NHE1 WT or non-phosphorylatable NHE1 at either Akt (S648A) or Erk1/2 (S770/771A) phosphosites, or a loss of function mutant (LOF, N266H3). To asses NHE1 localization, we used immunofluorescence (IF) labeling and a surface protein pulldown assay.

Results: NHE1i arrested RPE cells in the G0/G1 phase of the cell cycle. Inhibition of Akt, but not Mek, resulted in a decrease of pHc similar to NHE1i in RPE cells. A431 cells expressing NHE1 WT or S770/771A displayed an ~25% increase in growth/proliferation compared to NHE1 S648A or LOF. NHE1 WT, S648A, and S770/S771A were observed to localize to the cell membrane while NHE1 LOF remained intracellular in both IF and pulldown assay.

Conclusions: Phosphorylation of NHE1 by Akt does not impact NHE1 localization in cells but seems to increase cell growth/proliferation, likely by increasing pHc. Further confirmation of S648 phosphorylation by Akt in vivo is needed. Our current approach centers around the use of phospho-specific antibodies and patient samples as well as mass spectrometry. We aim to further study the regulation of NHE1 and its potential dysregulation as a driver of cancer growth.

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P9 Cytosolic pH regulates cell cycle progression and tumorigenesis by promoting expression of Cyclin D1

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Introduction: Enhanced cell growth and proliferation are accompanied by profound changes in cellular metabolism. Although originally identified as the Warburg effect in tumor cells, these changes are common also under physiological conditions and include increased dependence on fermentative metabolism and increased cytosolic pH[1, 2]. However, how these changes contribute to enhanced cell growth and proliferation is unclear.

Experimental: We present a multifaceted approach using primary fibroblasts, immortalized cell lines, and tissue samples to identify molecular mechanisms underlying pH-dependent cell proliferation. In particular, we use luciferase reporters and ChIP experiments to characterize pH-dependent transcription of Cyclin D1, and test disease significance of our data in tumor samples.

Results: Here, we demonstrate that NHE-dependent regulation of cytosolic pH is required in the G1 phase of the cell cycle to promote Cyclin D1 transcription and, consequently, cell proliferation. We identify elements within the Cyclin D1 promoter and several transcription factors that mediate pH-dependent transcription of Cyclin D1. Surprisingly, pH-dependent regulation of Cyclin D1 is independent of upstream signaling pathways, such as MAP-kinase signaling. Instead, cytosolic pH regulates a specific molecular interaction at the promoter to drive transcription. Similarly, in malignant pleural mesotheliomas (MPM), which are tightly associated with increased Cyclin D1/Cdk4 activity[3-5], high NHE1 expression and cytosolic pH correlate with increased Cyclin D1 levels and sensitivity to NHE1 inhibition.

Conclusions: Our data not only identify a molecular mechanism underlying pH-dependent proliferation, but also suggest that upregulation of NHE1 activity is a critical step in the development of MPMs. Thus, these data may help to understand mechanisms of cellular transformation underlying a variety of Cyclin D1-dependent tumors.

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P10 ECM composition, extracellular acidosis and metabolism collaborate to drive Vasculogenic Mimicry in PDAC CSCs

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Introduction: Vasculogenic mimicry (VM) is the ability of CSCs to express an endothelial-like phenotype and participate in tumor neovascularization via the formation of a blood-conducting, matrix-rich meshwork. We have previously reported that PDAC CSCs develop their vascular phenotype on Matrigel via of two interacting and coordinated factors: (i) the intrinsic over-expression of genes for endothelial factors and vascular receptors and (ii) the very high secretion of numerous pro-angiogenic/growth factors which support their high growth rate in-order-to form the vascular network1. As normal stem cell differentiation is regulated by NHE1-driven pH2, we studied the role of the tumor microenviroment pHe/NHE1 and of metabolism in regulating CSC VM.

Experimental: CSCs grown on ECMs of different composition and in different medium pHs (pHe) were treated with inhibitors of the NHE1 and of metabolism and their ability to perform VM was analyzed.

Results: The ability to VM structures was highest on 90% Matrigel and decreased as collagen I content of the ECM increased. In all ECM compositions, VM capacity of the CSCs increased stepwise with pHe acidification. In all conditions the CSCs capacity to perform VM was strongly reduced by inhibition of (i) the NHE1 with cariporide and (ii) inhibition of various metabolic steps by incubation with 2DG, DCA and phenformin.

Conclusions: In addition to the intrinsic regulation of VM by ECM composition-determined gene expression and secretome, VM is up-regulated by the acidic TME via NHE1 activity and by the glycolytic metabolism which is also pHi dependent.

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P11 Diversity in connexin-dependent inter-cellular diffusive coupling in colorectal cancer cell lines

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Introduction: Connexin (Cx) proteins assemble into gap junctions (GJ) and functionally couple cells (1). Under selection pressures that drive carcinogenesis, coupled clusters of cancer cells are likely to respond differently as compared to uncoupled cells. We therefore investigated how Cx coupling varies among colorectal cancer (CRC) cell lines.

Experimental: Gene expression patterns in a panel of >100 CRC lines were analysed using microarray data. Cell-to-cell communication was measured in a subset of representative cell lines by FRAP of calcein, a marker of diffusive coupling (2).

Results: The most highly expressed Cx genes were GJA1 (Cx43), GJB2 (Cx26), GJB3 (Cx31) and GJC1 (Cx45), of which GJA1 and GJC1 had a bimodal distribution among CRC lines, possibly indicating a mutation-related dependence, whilst the others had a unimodal distribution. Functionally, CRC cells could be grouped. Those with high GJB2/B3 but low GJA1 expression (e.g. DLD1, HT29, SNU1235, SW948) had modest to strong diffusive coupling. Lines expressing both GJA1 and GJB2 (e.g. LOVO, LS174T) were moderately coupled. Based on siRNA knockdown results, GJB2 is likely the dominant contributor to inter-cellular coupling in these lines. In contrast, lines with high GJA1 expression and low or absent GJB2/B3 (e.g. C10, CACO2) manifested a degree of coupling only in a subpopulation of cells.

Conclusions: GJB2, which is normally expressed in colonic epithelium, underpins inter-cellular coupling in a subgroup of CRC cell lines. Expression of other Cx genes may produce either no coupling or a heterogeneous pattern of connectivity. These differences may determine whether the unit of selection in cancer progression is an individual cell or a cluster of cells.

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P12 The N-terminally truncated isoform of Hv1 is overexpressed in tumorigenic human breast cell lines.

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The current knowledge indicates that neoplastic transformation is associated with a metabolic deregulation and acid overproduction [1]. In breast cancer cells, we proved that voltage-gated proton channel (Hv1) is functionally relevant in basal intracellular pH control. Comparing the effect of Hv1 inhibition in tumorigenic (MCF-7 and MDA-MB-231) and non-tumorigenic (MCF-10A) human breast cells we found, that it induced cycle arrest and cell viabiliy reduction without affecting MCF-10A cells. Here, we explore if these differences could be associated with the expression of the N-terminally truncated Hv1 isoform [HV1(S)] that is specifically enriched in malignant B cells resulting in higher proliferation and migration [2]HVCN1 associates with the B-cell receptor (BCR.

Experimental: we examined (by western blot and flow cytometry) the expression of the HV1 in the three human breast cell lines. We tested the amount of both isoform [Hv1(L+S)] using a polyclonal antibody which recognize the residues 32-44, present in both isoforms. Then, the large isoform [Hv1(L)] was detected using an antibody that recognize the residues 1-30 of the N-terminal region present only in the Hv1(L).

Results: we found an equal amount of Hv1(L+S) expressed among the three cell lines. However, the expression of Hv1(L) showed a signifcant decrease (44% and 46% in MCF-7 and MDA-MB-231 respectively vs MCF-10A cells $p<0.01$). Flow cytometry confirmed that Hv1(L) isoform is reduced (40% and 90 % in MCF-7 and MDA-MB-231 respectively vs the MCF-10A cells $p<0.01$). So, in both cases we can infer that such reduction represents the Hv1(S) amount.

Conclusions: we demonstrated for the first time that breast cancer cells overexpress the HV1(S) isoform in comparison with non-tumorigenic breast cells. This result could explain the mayor sensibility to Hv1 inhibition observed in tumorigenic cells, as well as points out the importance of evaluating the contribution of both isoforms to establish if the HV1(S) could be a more selective marker of cellular malignancy.

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P13 5-substituted amiloride derivative Na+/H+ exchanger (NHE) inhibitors potently induce cell type specific death of cancer cells in 3D culture, independent of NHE1

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Introduction: The high metabolic acid production of cancer cells renders them vulnerable to inhibition of acid-extruding transporters (1,2). While Na+/H+ exchanger-1 (NHE1) is an important regulator of cancer cell growth, NHE1 inhibitors can also exert NHE1-independent cytotoxic effects (3,4). The aim of this study was to compare effects of NHE inhibitors versus genetic ablation of NHE1 on growth and survival of cancer cells and investigate how these compounds induce NHE1-independent cancer cell death.

Experimental: Cancer (colon, breast and pancreatic) and non-cancer (mammary epithelial and mouse fibroblast) cell lines were grown as monolayers (2D) or as 3D spheroids and treated with amiloride-type (amiloride, EIPA, DMA, HMA) or benzoylguanidine type (eniporide, cariporide) for 2-7 days. Spheroids were subjected to viability assays, accumulation studies and western blotting for proliferation- and cell death markers, and 2D cultures were analyzed by immunofluorescence.

Results: EIPA, DMA and HMA elicited a strong, dose-dependent reduction in viability of breast cancer spheroids while cariporide and eniporide had no effect. Importantly, inhibitor-induced loss of viability was identical between WT cells and 2 NHE1 CRISPR/Cas9 KO clones. Inhibition of NHE-mediated Na+/H+ exchange was similar for both compound types in 2- and 3D culture. EC50 values were generally lower in cancer cells than normal cells. Extensive accumulation of EIPA and HMA was observed after long-term treatment, both in 2- and 3D culture. 48 h EIPA treatment elicited decreased proliferation, increased gH2AX levels, PARP cleavage, and mitochondrial damage.

Conclusions: Many commonly used NHE1 inhibitors exert potent, NHE1-independent cytotoxicity on cancer cells upon long-term treatment. While our data warrants against interpreting long-term effects of these compounds as indicative of roles of NHE1, the high sensitivity of cancer cells suggest a relevance of these compounds in anti-cancer therapy.

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P14 Screening in colorectal cancer: Proposal for a new improved protocol

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Introduction: Colorectal cancer (CRC) is the third most prevalent cancer and the fourth principal cause of cancer death in the world. Sporadic CRC arises from the accumulation of successive genetic and epigenetic abnormalities found in colonic cells that promote abnormal cellular growth, evasion of apoptosis, and differentiation. (1–6)
Many CRC deaths could be avoided through improved screening modalities and protocols. (3,7–12)
Due to the notorious heterogeneity of CRC, it is possible that optimal screening will consist of a sequential battery of blood and stool tests.(13–21)

Experimental: This work is the result of a comprehensive literature and our recent epigenetic studies on prostate, colon, urologic, lung and pancreatic cancers.

Results and conclusions: We propose to collect blood and stool from patients at the same time and test the stools with guaiac fecal occult-blood test/ fecal immunochemical test (gFOBT/FIT) to detect bleeding polyps. If the test is positive, patients should undergo colonoscopy with biopsy or other diagnostic technique. If negative, multi-target stool DNA test (MT-sDNA) should be used to detect eventual non-bleeding polyps (scaly polyps). At this stage if the test is positive, patients should perform diagnostic techniques, if negative the Septin9 blood assay should be done to detect eventual aberrant methylations on the vimentin promoter in the plasma. Again, if the test is positive there is indication for colonoscopy or other diagnostic technique. If this test is negative this indicates that a significant percentage of CRC histological presentations have already been excluded. However, it is still possible that a flat or incipient lesion is present. Therefore, the epigenetic studies on blood and stool currently underway might shed new light on the identification of these tumors.
It is possible that the described sequential procedures might have a strong impact on CRC screening as cumulative specificity and sensitivity would be at play.

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P15 SPINT2 as a therapeutic biomarker in Melanoma

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SPINT2 is a serine proteases' inhibitor identified as a tumor suppressor gene. SPINT2 promoter is hypermethylated in various solid tumors, including melanoma leading to its down regulation1 extracellular proteolytic regulation of HGF activation, which is influenced by the tumor microenvironment, and its consequential effects on melanoma malignancy remain uncharacterized. In this study, we identified SPINT2 (serine peptidase inhibitor Kunitz type 2. BRAF and MEK inhibitors are melanoma target therapies. Patients ultimately develop resistance possibly mediated by HGF secretion from tumor adjacent cells2. HGF is activated through serine proteases and is the only ligand known for MET receptor3. So, MET inhibitors can be a way to overcome this resistance. The evaluation of SPINT2 functional role and therapeutic potential in melanoma are the aims of this work.

We produced stable transfectants of melanoma cell lines to modulate SPINT2 expression; Performed functional studies in 2D/3D culture models; Evaluated molecules affected by SPINT2 deregulation; glucose consumption/lactate efflux; and tumor response to targeted therapies.
SPINT2 expression resulted in a decrease of cell viability, migration and proliferation; Higher levels of E-cadherin and lower levels of N-cadherin; Lower glucose consumption and lower extracellular lactate levels; Higher sensitivity to melanoma targeted therapies. Conversely, SPINT2 absence lead to a higher efficacy of MET inhibitors in melanoma cells.
In summary, our preliminary results confirmed that SPINT2 acts as a tumor suppressor gene in melanoma and that it has a potential value as a therapeutic biomarker for melanoma patients.

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P16 Epigenetic inhibitors to reprogram breast cancer metabolism through epigenetic changes

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Online publication not authorized.

P17 Crosstalk between cell cycle and metabolism

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Cell cycle and cell metabolism are two fundamental interlinked processes that sustain life. Core cell cycle regulators are shown to directly and indirectly regulate the activity of metabolic enzymes and vice versa (1). Even though there are numerous studies on this topic, the high variability in models and techniques make it difficult to conclude which are the main conserved mechanisms and their regulation. Therefore, the aim of our work is to study the crosstalk between glucose metabolism and cell cycle regulation in a non-transformed human cell line.

In this study, we used Fluorescence Ubiquitin Cell Cycle Indicator (FUCCI) system to visualize the progression of different cell cycle phases (2). We combined the FUCCI system with Fluorescence Resonance Energy Transfer sensors to follow the oscillations of metabolites such as glucose, ATP and lactate during cell cycle (2, 3). We performed live confocal microscopy with temperature and CO2 control, which allows the analysis of the metabolic fluctuations under unperturbed condition. In addition to that, we followed the changes in mitochondrial morphology and mitochondrial redox-state during the cell cycle.

Our results show that glucose, ATP and lactate levels oscillate during the cell cycle. Interestingly, perturbations in mitochondrial morphology lead to disturbed cell cycle progression and G1-phased mitochondria redox-state is more oxidized compared with the other phases. In fact, altered cellular redox balance can perturb normal cell cycle progression.

All together our results suggest that during the cell cycle there are metabolism fluctuates to respond to the different cellular demands. Our findings in a non-transformed model are a starting point to understand how metabolic alterations can lead to uncontrolled proliferation in cancer and vice-versa.

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P18 Nrf2 regulates the expression of the TP53-Induced Glycolysis and Apoptosis Regulator in cancer cells

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Introduction: TP53-Induced Glycolysis and Apoptosis Regulator (TIGAR) was described as a p53 response gene induced by ionizing radiation1,2. However, TIGAR is overexpressed in many tumors independently of p53 levels, where it acts as a bisphosphatase on fructose-2,6-P2, increasing the pentose phosphate pathway in a cell-specific manner.
The nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a master orchestrator of the antioxidant response. In cancer cells, Nrf2 can be constitutively activated through the PI3K/Akt signaling pathway, which we previously demonstrated that controls TIGAR protein levels3, and confers chemo- and radio-resistance.

Experimental: Nrf2 was induced and inhibited in HeLa cells through different approaches. The expression of TIGAR and several known Nrf2 target genes was determined, and luciferase assays were performed to characterize the binding of Nrf2 to TIGAR promoter.

Results: Treatment of HeLa cells with the Nrf2 inducer dimethyl fumarate enhanced TIGAR expression in a transcription-dependent manner. Nrf2 overexpression increased TIGAR mRNA and protein levels, and siRNA-mediated inhibition of Nrf2 resulted in decreased TIGAR expression and protein amount. Functional analyses of TIGAR promoter revealed increased luciferase activity of a construct containing two antioxidant response elements corresponding to the consensus Nrf2 binding sequence.

Conclusions: Nrf2 is involved in the transcriptional control of TIGAR in HeLa cells, providing a p53-alternative mechanism for TIGAR overexpression in those tumours with high oxidative stress.

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P19 Effect of microenvironment on response to L-asparaginase treatment in leukemia cells

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Introduction: L-asparaginase (ASNase) is the key component in the treatment of acute lymphoblastic leukemia (ALL) in children. ASNase depletes extracellular asparagine (Asn) and glutamine (Gln), considering them semi-essential amino acids (AAs) in cancer cells. The mechanism responsible for different sensitivity to ASNase in leukemia patients is not completely known. We have previously shown that effectiveness of ASNase is decreased by rewiring of cellular metabolism. Aim of this study was to investigate how microenvironment of bone marrow influences metabolic setup of leukemia cells and how it interferes with response to ASNase treatment.

Experimental: Precursor-B ALL cell lines (Nalm-6, Reh) were cultivated in the absence or presence of mesenchymal stem cells (MSCs) both in normoxia and hypoxia. Sensitivity of leukemia cells to ASNase was determined at different time-points by flow cytometry. Concentration of extracellular AAs from the culture media was assessed by HPLC. Glucose uptake and fatty acid oxidation measurements were performed by radioactivity assays.

Results: Presence of MSCs increased survival of leukemia cells upon ASNase treatment both in normoxia and hypoxia. The uptake of most essential and non-essential AAs was increased in the co-culture with MSCs. Preliminary measurements suggested changes in metabolic activity of leukemia cells upon ASNase treatment in co-culture with MSCs.

Conclusions: Our preliminary data show that MSCs rescue leukemia cells by change of AAs content in media which is partially supported by previous data (1). Preference for bioenergetic pathways is altered in the presence of MSCs. Hypoxia does not impair effect of MSCs on leukemia cells' survival after ASNase treatment.

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P20 Functional and prognostic relevance of CDH3/P-cadherin in glioblastoma

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Introduction: Glioblastoma (GBM) is the most common and malignant primary brain tumor in adults, with a median overall survival of 15 months1. P-cadherin is an adhesion molecule described to act as a tumor promoting or suppressor molecule, according to the molecular and cellular context2. As nothing is known about the relevance of this classical cadherin in GBM, here we investigate its functional roles and prognostic relevance.

Experimental: CDH3/P-cadherin expression was evaluated at the mRNA and protein levels in GBM samples. Functional roles on cell viability (trypan blue), invasion (3D spheres) and stemness capacity (neurospheres) were tested in vitro using silencing and overexpressing approaches in a GBM primary culture and GBM cell line, respectively. In vivo subcutaneous and orthotopic xenograft models were established in immunodeficient hairless NOD.SCID and NSG mice, respectively. Gene Set Enrichment Analyses were performed in TCGA GBM patients. CDH3 expression impact in patients' prognosis was evaluated with Log-rank, Cox multivariate analyses and meta-analysis.

Results: P-cadherin expression is increased at mRNA and protein level in a subset of high-grade gliomas. In vitro, P-cadherin is associated with increased viability, invasion and stemness capacity of GBM. In vivo, cells overexpressing P-cadherin form larger subcutaneous tumors and associated with shorter mice survival when orthotopically injected. Mechanistically, CDH3 positively correlates with genes involved in cancer-related pathways. At the clinical setting, GBM patients from various cohorts expressing high levels of CDH3 present shorter overall survival.

Conclusions: We show CDH3/P-cadherin is associated with GBM aggressiveness and patient prognosis, suggesting it as a therapeutic target. Future studies will explore how P-Cadherin may affect critical energetic metabolic pathways in GBM.

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P21 WNT6 is a novel oncogenic prognostic biomarker in human glioblastoma

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Introduction: Glioblastoma (GBM) is a universally fatal brain cancer, for which novel therapies targeting specific underlying oncogenic events are urgently needed. While the WNT pathway has been shown to be frequently activated in GBM, constituting a potential therapeutic target, the relevance of WNT6, an activator of this pathway, remains unknown.

Experimental: WNT6 protein and mRNA levels were evaluated in GBM. WNT6 levels were silenced or overexpressed in GBM cells to assess functional effects in vitro and in vivo. Phospho-kinase arrays and TCF/LEF reporter assays were used to identify WNT6-signaling pathways, and significant associations with stem cell features and cancer-related pathways were validated in patients. Survival analyses were performed with Cox regression and Log-rank tests. Meta-analyses were used to calculate the estimated pooled effect.

Results: We show that WNT6 is significantly overexpressed in GBMs, as compared to lower-grade gliomas and normal brain, at mRNA and protein levels. Functionally, WNT6 increases typical oncogenic activities in GBM cells, including viability, proliferation, glioma stem cell capacity, invasion, migration, and resistance to temozolomide chemotherapy. Concordantly, in in vivo orthotopic GBM mice models, using both overexpressing and silencing models, WNT6 expression was associated with shorter overall survival, and increased features of tumor aggressiveness. Mechanistically, WNT6 contributes to activate typical oncogenic pathways, including Src and STAT, which intertwined with the WNT pathway may be critical effectors of WNT6-associated aggressiveness in GBM. Clinically, we establish WNT6 as an independent prognostic biomarker of shorter survival in GBM patients from several independent cohorts.

Conclusions: Our findings establish WNT6 as a novel oncogene in GBM, opening opportunities to develop more rational therapies to treat this highly aggressive tumor. Future studies are warranted to explore how WNT6 may associate with particular metabolic profiles in GBM.

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P22 Clinical Implications of the Autophagy Core Gene Variations in Acute Myeloid Leukemia

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Introduction: Acute myeloid leukemia (AML) comprises a heterogeneous group of neoplasms characterized by an impaired differentiation and/or proliferation of immature progenitor's cells. AML has still an aggressive clinical course and limited chemotherapy options. Considering the essential role of autophagy in AML cells response to chemotherapy, we hypothesized that genetic variants in autophagy core genes might contribute to outcomes of AML.

Experimental: We examined the role of single nucleotide polymorphisms (SNP) in autophagy-associated genes (ATG10 rs1864182, ATG16L1 rs2241880, IDH1 rs11554137, PSBM9 rs17587, AMPK2a rs3738568, AMPK3a rs64336094, AMPK3a rs692243, and IRGM rs72553867) in 284 patients with AML from a Spanish cohort and in 324 healthy individuals.

Results: The results showed that ATG10 rs1864182 TT genotype (OR=1.66; 95% CI 1.16-2.37; p=0.006), AMPKa2 rs3738568 TC genotype (OR=2.40; 95% CI 1.01-5.69; p=0.021) and IDH1 rs11554137 T allele carriers (OR=1.58; 95% CI 1.03-2.43; p=0.036) are associated with increased risk of AML development. Logistic regression adjusted for age and gender confirm the association of ATG10 rs1864182 TT genotype (OR=1.68; 95% CI 1.10-2.58; p=0.016) and IDH1 rs11554137 T allele carriers (OR=1.74; 95% CI 1.04-2.91; p=0.034) with increased risk of AML development. Similar association as found for ATG16L1 rs2241880 T allele carriers (OR=1.53; 95% CI 1.00-2.34; p=0.0048). The stratification of the analysis according to the AML subtypes show that different SNPs may be associated with different subtypes.

Conclusions: These results disclose that autophagy core gene variations may impact on the regulation of AML development.

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P23 Using an *ex vivo* Platform to Target Metabolic Heterogeneity and Improve Chemotherapeutic Efficacy in High Grade Serous Ovarian Cancer

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Introduction: Clinical attempts to target tumour metabolism are encumbered by approaches that do not factor in the metabolic heterogeneity of patient cohorts. Herein we present a method that enables the rapid assessment of metabolic targeting in an ex vivo patient context. Focusing on High Grade Serous Ovarian Cancer (HGSOC), we aim to show that the addition of common inhibitors of glycolysis (2-deoxy-d-glucose) and oxidative phosphorylation (atovaquone and metformin) to standard of care chemotherapy carboplatin can increase therapeutic efficacy based on patient metabolic preference.

Experimental: We have developed an explant culture system whereby patient tumour can be sliced reproducibly to maintain architecture and viability, with a short-term culture system through which we can rapidly assess therapeutic efficacy. Within this system we have developed a multi-pronged approach to assay tumour glycolysis and oxidative phosphorylation, featuring patient scans, protein expression, and immunofluorescent modalities. To our explant slices we are assaying the efficacy of drug combinations to delineate patients in terms of metabolic susceptibilities.

Results: We have thus far shown that low doses of antimetabolites alongside chemotherapy carboplatin garner in vitro synergistic efficacy in models of glycolytic and oxidative HGSOC. We have also shown that our explant culture system can be used to successfully elucidate tumour metabolic preferences and quantify the efficacy of drug treatments based on antiproliferative and cytotoxic effects. Current efforts are being directed towards defining these preferences in a HGSOC patient cohort.

Conclusions: Metabolic targeting is a promising therapeutic avenue but is encumbered by approaches that do not sufficiently consider metabolic heterogeneity. The present work aims to address this gap and provide a means whereby metabolic targeting can be assessed in heterogeneous patient cohorts.

P25 Heterogeneous expression of genes associated with cancer pathway in selected brain tumors

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Introduction: Primary brain cancer, especially glioblastoma multiforme (GBM) is one of the most aggressive and difficult-to-treat malignancies of Central Nervous System (CNS). For this reason, we have undertaken to determine the profile of genes involved in the process of tumorigenesis among various types of brain tumors: meningiomas, astrocytomas and glioblastomas.

Experimental: For molecular analysis, we used a pre-designed Human Cancer PathwayFinderTM PCR Array to quantify the expression of 84 genes associated with different cancer pathways. The quantitative PCR was performed on the ViiA7 Real-Time PCR system and used SYBR Green detection. The difference in gene expression between control (commercial RNAs from normal brain cells, n= 3) and test group of samples was performed by ΔΔCt method.

Results: We determined significant changes in all signalling pathways (n= 9) between control and tumor groups, while the most pronounced deregulation (≤ -2.0 or +2.0) were observed in 30% events. The most gene expression changes (n= 44) from the tumor group were detected in astrocytomas. Conversely, in cases of aggressive types of glioblastomas, we did not detect higher levels of genes compared to a low grades. Interesting was the achievement of slight changes in apoptotic genes and downregulation of the main genes responsible to metabolism. In tumor samples, we have determined a high deregulation of the following genes: CA9, FLT1, GSC, MAP2K1, MKI67, PGF, SLC2A1, SOX10, STMN1 and WEE1.

Conclusions: The results of this study underline the importance of molecular signalling in heterogeneous tumor tissue. The identification of the cancer pathway genes in biopsies from patients may help improve the effectiveness of treatment and may broaden our understanding of CNS cell metabolism.

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P26 Mitochondrial alterations associated to cisplatin resistance in ovarian cancer

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P27 Delving into connection of LDH-B and mitochondria as an alternative AML treatment

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Online publication not authorized.

P28 The expression of monocarboxylate transporter 4 unravels novel pathologic mechanisms in giant cell tumor of bone

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Introduction: Giant cell tumor of bone (GCTB) is a highly recurrent benign, albeit aggressive, primary bone tumor. GCTB is characterized by the presence of giant multinucleated osteoclast-like cells that abnormally resorb bone, mixed with proliferating spindle-shaped cells carrying a specific mutation of histone 3.3 with an unclear function and that, recently, have gained a major interest. Remarkably, in GCTB, a strong avidity for FDG has been reported, even higher than malignant bone cancers [1], thereby suggesting a high glycolytic activity for this tumor. However, the metabolic profile of the GCTB has never been explored.

Experimental: We analyzed the expression of glycolytic markers and of the monocarboxylate transporter 1 and 4 (MCT1 and MCT4) in GCTB tissues and GCTB isolated cells by Q-RT-PCR, ELISA or immunostaining. We also quantified lactate in tumor tissues and in serum samples of patients.

Results: We found a high expression of MCT4, both at mRNA and protein level in GCTB tissues, which directly correlates with the intratumor lactate levels. We also found that the high glycolytic metabolism in GCTB is mainly confined in osteoclast-like cells, whereas the spindle-shaped cells are mainly characterized by an oxidative basal metabolism. Furthermore, we found that high serum lactate (>3.035 mM) is predictive of local or systemic tumor relapse in GCTB patients, suggesting that lactate might be considered as a predictive marker of GCTB recurrence.

Conclusions: We unrevealed a novel pathogenic mechanism of GCTB by enlightening the strongly glycolytic nature and the MCT4 expression that offers as novel opportunities for therapeutic intervention.

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P29 Non-conformable metabolic responses to limiting nutrient conditions by different cancer cells

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Introduction: Metabolic imaging for cancer diagnostic and response to chemotherapy requires fundamental understanding of metabolic plasticity in a precarious nutrient microenvironment. An approach for experimentally discerning metabolic pathways is 13C-glucose-derived metabolite profiling in a well-defined culture system consisting of medium with limiting glucose / glutamine combinations. The diversity of metabolic activities in different tumor cells can be mimicked by comparing the breast cancer cell lines MCF-7 (low malignancy) and MDA-MB231 (high malignancy).

Experimentals: Cells were cultured as monolayers in medium containing either standard DMEM or combinations of glucose (1.0; 2.5 mM) and glutamine (0.1; 1 mM). After a 3-day conditioning period, cells were labeled with [U-13C] glucose for 2h and analyzed by gas chromatography / mass spectrometry. Conversion of hyperpolarized [1-13C] pyruvate to 13C-lactate was performed with suspended cells using NMR.

Results: Metabolic differences of these tumor cells depended on the glucose / glutamine combination, as shown by changes in the 13C-isotopologue profiles of pyruvate, lactate, alanine and serine, which indicate both 13C-glucose-derived de novo synthesis and replenished 13C-pyruvate (with reduced 13C-label). TCA-metabolites denote that pyruvate anaplerosis was higher in MDA-MB231 than in MCF-7 cells, but only in limiting glucose conditions. The utilization of replenished pyruvate for lactate and alanine conversion was much preferred by MDA-MB231 cells, being pronounced in the most precarious but low in saturating glucose / glutamine conditions. Unexpectedly, the conversion rate of 13C-pyruvate to 13C-lactate was about 2-fold lower in MDA-MB231 than in MCF-7 cells, correlating with a higher intracellular lactate pool.

Conclusion: The results illustrate cancer cell differences in metabolic fine tuning when in a precarious nutrient environment and point to complex dynamics of metabolic states in a solid tumor in vivo.

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P30 Cisplatin-resistant urothelial bladder cancer cells undergo metabolic reprogramming

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Introduction: Advanced urothelial bladder cancer (UBC) patients are tagged by a dismal prognosis and high mortality rates, mostly due to their poor response to standard-of-care platinum-based therapy 1. Mediators of chemoresistance are not fully elucidated 2. We aimed to study glucose metabolism in advanced UBC, in the context of cisplatin resistance.

Experimental: Two isogenic pairs of parental cell lines (T24 and HT1376, ATCC) and the matching cisplatin-resistant (CR) sublines (Resistant Cancer Cell Line collection) were used. A first screening on glycometabolism was performed by Western-blot, immunofluorescence (glycometabolism biomarkers) and colorimetric kits (glucose consumption, lactate and ATP production). Flow cytometry analysis was used to evaluate glucose uptake, mitochondrial activity, ROS production and PD-L1 levels. Cellular oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured using the Seahorse XFe96 Extracellular Flux Analyser.

Results: In comparison to the parental sublines, T24-CR and HT1376-CR exhibited higher dependence on glucose, higher extracellular lactate levels and lower ATP production; no differences in mitochondrial activity or ROS production were noted. The glycolytic phenotype was particularly evident for HT1376/HT1376-CR pair, for which the cisplatin-resistance ratio was higher. Regarding OCR profile, HT1376-CR cells showed decreased basal respiration and oxygen consumption associated to ATP production; in accordance, ECAR was also higher in the resistant subline. Glycolytic rate assay confirmed that these cells presented higher basal glycolysis, with an increase in proton efflux. Interestingly, cisplatin-resistant cells presented decreased PD-L1 levels.

Conclusions: Cisplatin-resistant UBC cells seem to display an increased glycolytic metabolism. Therefore, glycometabolism may harbour potential targets to overcome therapy resistance. Further studies are needed to unravel the underlying molecular mechanisms.

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P31 Reactive Oxygen Species (ROS) a motor in Endothelial Cells (ECs) activation

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Introduction: The increased generation of reactive oxygen species (ROS) is a hallmark of cancer cells metabolic remodeling.1,2 Despite ROS has been linked to toxicity and cell dysfunction, it is known that, depending on the cell type, low ROS levels can act as regulators of signaling pathways.2 Thus non-cancerous cells in tumor microenvironment (TME) can be modulated by ROS in order to contribute for cancer progression. Endothelial cells (ECs) metabolism was not extensively addressed so far. Thus, giving the crucial relevance of angiogenesis in cancer progression, we investigated the role of ROS in endothelial activation and sprouting, together with the influence of cysteine; serving as a ROS scavenger, directly or as a precursor of glutathione4,5,6,7.

Experimental: ***In vitro*** Human Umbilical Vein Endothelial Cells (HUVECs) and ***ex vivo*** Wistar rat (Rattus norvegicus) aortic rings were used to test the role of ROS (hydrogen peroxide-H2O2; 15 μM) and cysteine (400 μM) in ECs activation and sprouting. Proliferation, migration and sprouting capacities were tested in HUVECs by respectively flow cytometry (cell cycle; propidium iodide-PI labeling), wound healing assay and matrigel tube forming assay. The spouting capacity of rats aorta was tested in matrigel embedded aortic rings assay. By qPCR the expression profile of endothelial related genes was assessed in the tested culture conditions.

Results: H2O2 activates ECs in HUVECs and aortic rings, by increasing proliferation, migration and sprouting. Interestingly, cysteine partially abrogates these effects. The mRNA expression profile in HUVECs varies according to culture conditions, fitting the modulation of ECs by H2O2 and cysteine.

Conclusions: ROS is a relevant regulator of ECs activation, ***in vitro*** and ***ex-vivo***. Our results suggest that in TME, the increased ROS levels by cancer cells may induce a metabolic remodeling of ECs, accounting for their activation, in order to prompt angiogenesis and favor tumor progression.

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P32 Study of metabolic heterogeneity of tumors at clonal level and its modulation by anti-angiogenic therapy

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Introduction: Cancer is associated with metabolic changes, including dysregulated glucose metabolism. Whether this event depends on the presence of tumor sub-populations with different metabolic features or local modulation of the metabolism associated with microenvironment (i.e. hypoxia) has less been investigated[1]. We previously reported that anti-VEGF therapy induces a stable metabolic change in ovarian cancer xenografts that correlates with tumor aggressiveness and resistance to therapy[2].

Experimental: We isolated clones from human ovarian cancer cell lines previously characterized for their glycolytic activity and tested their viability following 48-72 h glucose starvation. We thus identified Glucose Deprivation Sensitive (GDS) and Glucose Deprivation Resistant clones (GDR). We studied by a multimodal approach (functional metabolic assays, untargeted MS, whole-exome and transcriptome analysis) key metabolic features of GDR and GDS clones both under normal culture conditions and under glucose starvation.

Results: We found a great heterogeneity in percentage of GDR and GDS clones in the 6 ovarian cancer cell lines tested (IGROV-1, OC316, SKOV3, A2780, A2774 and OAW42). Although glycolytic activity was comparable in GDR and GDS clones, the latter showed reduced OXPHOS activity. Importantly, although the percentage of GDR and GDS clones obtained from tumor xenografts was similar to that found in parental cell lines, cultures established from anti-VEGF treated tumors showed marked enrichment in GDR clones.

Conclusions: Overall, we found that ovarian cancer cell lines are composed by different proportions of GDS and GDR clones. Moreover, anti-VEGF therapy is able to skew the GDR/GDS ratio in tumor xenografts, favouring accumulation of GDR clones. Drugs specifically targeting GDR clones could be used to overcome resistance to anti-VEGF therapy.

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P33 Serum free amino acids in head and neck cancers as a promising prognostic marker

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Introduction: Despite distinctive advances in the field of head and neck squamous cell cancer (HNSCC) biomarker discovery, the spectrum of clinically useful prognostic serum biomarkers is limited. As metabolic activities in highly proliferative transformed cells are fundamentally different from those in non-transformed cells, specific shifts in concentration of different metabolites may serve as diagnostic or prognostic markers. Blood amino acids have been identified as promising biomarkers in different cancers before, but little is known about this field in HNSCC.

Experimental: Blood amino acid profiles of 140 HNSCC patients were examined using high-performance liquid chromatography. Cox proportional hazards regression model was used to assess the prognostic value of amino acid concentrations in serum. Colony forming assay was used to identify the effect of amino acids that were significant in Cox proportional hazards regression models on colony forming ability of FaDu and Detroit 562 cell lines.

Results: In the multivariable Cox regression model for overall survival (OS), palliative treatment was associated with an unfavourable prognosis while high serum levels of methionine have had a positive prognostic impact. In the relapse-free survival (RFS) multivariable model, methionine was similarly identified as a positive prognostic factor, along with tumor localization in the oropharynx. Oral cavity localization and primary radio(chemo)therapy treatment strategy have been linked to poorer RFS. 1mM serine was shown to support the forming of colonies in both tested HNSCC cell lines. Effect of methionine was exactly the opposite.

Conclusions: In this study, we hypothesize that minimally invasive metabolomic approach may provide a relevant method to distinguish clinically important subgroups between HNSCC patients. This study aimed to quantitate free amino acids in human serum and identify potential HNSCC biomarkers.

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P34 Role of RKIP protein in the modulation of cancer cells metabolism in solid tumors

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P35 CATALASEing Fanconi Anemia head and neck cancer cell death

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P36 Role of TIGAR in lymphocyte activation and proliferation

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Introduction: TP53-Inducible Glycolysis and Apoptosis Regulator (TIGAR) is a gene which has been described to be overexpressed in several types of tumors and to have a role in the metabolic switch in cancer cells. For that reason, it has been thought that its function could be related to physiological proliferation. In this project, we analysed the role of TIGAR in the proliferation of primary human T lymphocytes, which rapidly undergo mitosis after their activation.

Experimental: The first aim of this project was to determine if there was any variation in TIGAR levels after lymphocyte activation using concanavalin A (ConA) and lipopolysaccharide (LPS) and to analyse the location of this protein during T cell proliferation. The results showed that there is a rise of TIGAR expression in treated lymphocytes compared to control ones, and that TIGAR is located in the cytoplasm. Next, we analysed the signalling pathway responsible for the activation of this protein.

Results: The results showed that in the presence of PI3K/AKT pathway inhibitors, TIGAR induction was prevented. Finally, we determined the function of TIGAR in T cell proliferation. The results obtained suggested that TIGAR is involved in leading carbon flux to the pentose phosphate pathway (PPP) at the expense of glycolysis. At the same time, TIGAR probably protects the cell from oxidative stress and prevents autophagy, measured by p62 protein levels.

Conclusions: In conclusion, our results demonstrate that TIGAR plays a role in human physiological lymphocyte activation and proliferation through the PI3K/AKT signalling pathway. What is more, these results show a possible connection between TIGAR and several functions which are essential for cell proliferation.

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P37 Redox profiles of mitochondrial breast cancer subtypes

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Introduction: The capacity of tumour cells to growth and disseminate are profoundly influenced by the adaptation of their metabolism to extra- and intracellular oncogenic signals. This property underlies the heterogeneity of tumour subtypes and the development of chemoresistence. Here, mitochondrial breast cancer subtypes, previously identified in our laboratory, were characterized for their redox profiles. We focused on glutathione and thioredoxin systems, directly involved in the modulation of thiol-redox signalling.

Experimental: MCbiclust (1) was used for breast cancer sample stratification [TCGA (2) and METABRIC (3)] according to mitochondrial gene expression profiles. On breast cancer cell lines, we evaluated mitochondrial function, metabolic profile by functional imaging, biochemical approaches and mass spectrometry. The thiol-redox profile was analysed by quantification of total thiol groups, glutathione concentration and redox state, together with measurements of enzymatic acivity and protein levels of the glutathione and thioredoxin systems.

Results: We have found that the mitochondrial subtypes were characterised by differential glutamine utilisation, associated with adaptive changes in mitochondrial function. Moreover, we found specific redox properties of the mitochondrial subtypes. Intriguingly, the glutamine addicted mitochondrial subtype was more susceptible to oxidative stress following glutamine restriction, as shown by the decrease of thiol levels and concomitant increased glutathione oxidation. Enzymatic activities and protein expression analysis carried out on glutathione and thioredoxin systems also revealed different pattern of enzymes involved in cellular redox control in the mitochondrial breast cancer subtypes.

Conclusions: Metabolic gene expression profiling has stratified breast cancer patients into mitochondrial subtypes. These subtypes had different redox phenotypes, which could be exploited to develop new personalized cancer treatments.

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P38 ECM internalization as a novel modulator of breast cancer cell growth

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Introduction: Altered cellular metabolism is one of the hallmarks of cancer. Due to tumour growth rate and limited blood supply, the tumour microenvironment is often hypoxic and deprived of nutrients (including glucose and amino acids) [1]. Cancer cells have been shown to use extracellular protein to support nutrient signalling and cell growth; however, the contribution of a 3D extracellular matrix (ECM) and its endocytosis to cancer cell growth and metabolism is unclear.

Experimental: Here we investigated the ability of different types of ECM to support breast cancer cell growth under nutrient deprived conditions and we used a metabolomic approach to characterize the impact of ECM endocytosis on cell metabolism.

Results: Our data indicate that the presence of a collagen I or matrigel matrix is able to rescue breast cancer cell (but not non-transformed mammary epithelial cell) growth under glucose or amino acid deprived conditions. Interestingly, while the ECM supports cell proliferation in amino acid-depleted conditions, it seems to prevent apoptosis under glucose deprivation, indicating that the ECM exerts different roles depending on the starvation conditions. Moreover, the ECM rescues mTOR activation under amino acid and glucose starvation. Finally, metabolomics analysis indicate that components of the TCA cycle and amino acid metabolism are upregulated in amino acid deficient media in the presence of a collagen I matrix.

Conclusions: Our data indicate that breast cancer cells, but not normal epithelial cells, are able to use the ECM as an unconventional nutrient source, supporting cell growth under nutrient deprived conditions.

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P39 Low Cost and Ultrasensitive SERS-Based Paper Biosensor for cell type discrimination

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Introduction: Key enabling technologies (KETs) like Nanotechnology, surface-enhanced Raman scattering (SERS) spectroscopy and microfluidics have been prospering over the last years [1]. SERS is an ultrasensitive sensing technique, that overcomes the intrinsic low efficiency of Raman by using nanoparticles [1,2]. The high sensitivity of this analytical tool allows it to be used in chemical and biological analysis of analytes, even at a very low limits of detection [3].
However, one of the main challenges of the field has been the development of simple, ready-to-use and low-cost SERS substrates. Here, we have developed a portable, low-cost and scalable sensor based on SERS for the rapid identification of cell profiles, more specifically identify different biochemical components and molecular structures, such as lipids, nucleic acids and proteins.

Experimental: First, to control the fabrication of the SERS substrates, the intrinsic reproducibility of microfluidics technology was used for the fabrication of self-assembled nanoparticle structures over a paper film [4]. The paper substrates were fabricated by assembling anisotropic particles, gold nanostars (GNSs) and nanorods (NRs) onto paper to offer an extra enhancement to reach ultrasensitive detection limits. Thus, GNSs and NRs were synthesised by following conventional protocols with few modifications [5,6]. A polymer-paper hybrid device was used for the self-assembly of nanoparticles, which provided the ideal environment to control the drying kinetics of nanoparticles over the paper substrate. This method allowed a high reproducibility and homogeneity of the fabrication of SERS substrates that reached limits of detection down to the picomolar range and this method is quite simple and fast, takes less than 30 minutes and allows the preparation of several substrates simultaneously.

Results and Conclusions: A proof-of-concept experiment for the discrimination of two different cell populations, was designed to demonstrate the potential of this SERS on paper substrates for on-site detection of biochemical products which reveal additional and relevant information. The SERS spectrum of PBMCs vs. SW480 showed a significantly different profile. The prominent Raman peaks of the SW480 cells, compared with the PBMCs, demonstrated that there was an increase in the amount of proteins, as expected for cancer cells [7].
In conclusion, this work presents a novel strategy for the fabrication of a SERS paper-based sensor with high sensitivity, with high homogeneity and reproducibility. The proof-of-concept experiment allowed the differentiation of different cell populations by paving the way toward the development of advanced diagnosis tools based on nanotechnology.

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P40 Role of RKIP protein in the modulation of cancer cells metabolism in solid tumors

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P41 Fatty acid oxidation inhibition sensitizes prostate cancer cells to HSP90 inhibitor luminespib

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P42 Multi-omics approaches unveil novel sensitivities of ovarian cancer (OC) against fatty acid synthase (FASN) inhibitors

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Introduction: OC progression is dependent on lipid metabolism. Accordingly, OC cells overexpress FASN, the crucial enzyme of de novo lipid synthesis. It is known that FASN inhibitors abrogate OC growth and survival. However, in-depth analyses of the consequences of FASN blockade on the biocybernetic regulatory balance in OC cells are still missing [1].

Experimental: Here, a systems biology approach was applied combining 1) tandem mass spectrometry(MS/MS) shotgun proteomics followed by MaxQuant evaluation with 2) antibody microarray-based kinomics and with 3) multiple reaction monitoring(MRM)MS/MS targeted metabolomics. SKOV3 cells were cultured for 8h or 24h +/- 40µM FASN inhibitor G28UCM before lysis/analysis. Gene functional classification on the DAVID platform combined with KEGG pathway annotation was used to distinguish early E-, late L-, and sustained S-responses.

Results: E-responses included activated stress pathways (ER stress, unfolded protein response), apoptosis and autophagy, as well as inhibited nucleoside-, lipid- and central carbon-metabolism, including respiratory chain and electron transport. L-responses comprised inhibited DNA replication, ribosome formation, cytoskeleton- and chromatin-remodeling. S-responses included blockade of signaling, expression, transport, proteasome degradation and OXPHOS. Overall, metabolism and signaling responded prior to generalized stress response and protein downregulation. This correlated with loss of mitochondrial, membrane and signal lipids, amino acids, biogenic amines and monosaccharides, regardless of the presence of unlimited nutrients in the culture medium analogous to ascites.

Conclusions: In summary, membrane integrity was immediately compromised by the FASN inhibitor, resulting in serious defects in the uptake and transport of molecules. This indicates that the cells can not compensate for their nutrient deficiency by increasing the import of exogenous nutrients. This exquisite dependency of OC to lipogenesis and nutrient uptake should be exploited for local adjuvant/palliative therapy.

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P43 The potential of Ebastine in cancer treatments

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Introduction: Ebastine is a second generation antihistamine, commonly used for the treatment of allergies due to its efficacy, limited side effects and accessible costs. More recently, Ebastine has shown to be very effective killing cancer cells. In vitro, in vivo and epidemiological data support its anti-cancer properties and currently encouraged clinical trials in prostate cancer patients.

Experimental: Due to its amphiphilic nature, it is expected for Ebastine to accumulate in acidic tissues and compartments, such as tumors and lysosomes of cancer cells. In order to study the pharmacokinetics of Ebastine in different tissues after its oral administration, we used liquid chromatography coupled to mass spectrometry techniques (LC-MS). Additionally, we performed shotgun lipidomics analyses in tissues for investigating in what extent the use of Ebastine disturbs the lipid metabolism of tumor cells. These alterations are closely related to apoptotic pathways.

Results: Pharmacokinetics studies through LC-MS have shown higher levels of Ebastine in tumors than in other studied tissues after repeated doses. In accordance to in vitro experiments, shotgun lipidomics data from tumor tissues show specific alterations in the levels of cholesterol, sphingomyelin and ceramides, associated to lysosomal dysfunction and cancer cell death.

Conclusions: Taken together, these data suggest that Ebastine can be used for the development of new cancer strategies. This could bring relevant benefits to cancer patients, such as diminished side effects, more accessibility due to treatment costs and a lower mortality rate.

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P44 P-cadherin induces anoikis resistance in breast cancer stem cells by rewiring the metabolic flux and activating of antioxidant systems

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Introduction: Breast cancer stem cells (BCSC) exhibit a pro-glycolytic metabolism, allowing them to decrease oxidative stress, to escape anoikis and to survive in circulation[1]. P-cadherin (P-cad) is a poor prognosis factor in breast cancer, associated with hypoxic, glycolytic and acidosis markers[1,2]. Still, P-cad enriched populations, with increased stem-like properties, are more likely to exhibit increased glycolysis and to survive to metabolic-driven pH alterations[1,2]. The aim of this work was to evaluate if P-cad expression controls the metabolic program of BCSC, acting as an antioxidant and enhancing their survival in circulation by promoting anoikis-resistance.

Results: We found that P-cad expression regulates PDK4-induced PDH phosphorylation in P-cad enriched BCSC. Moreover, DCA induces anoikis preferentially in this BCSC population. We also demonstrated that P-cad decreases the oxidative stress, increases the antioxidant power as well as increases G6PDH expression, suggesting an enhanced metabolic flux through the antioxidant PPP in P-cadherin enriched BCSC. Additionally, P-cad expression upregulates SOD2 expression in BCSC. Importantly, this association was also validated in primary invasive breast carcinomas, where an enrichment of SOD2 expression was found in P-cad overexpressing breast carcinomas.

Conclusions: We demonstrate for the first time, that P-cad is responsible for the metabolic behavior BCSC via pPDH/PDK4 axis, as well as it modulates the oxidative stress of BCSC, through a redirection the metabolic flux into PPP as well as through an increase of SOD2 antioxidant system. The antioxidant role attributed to P-cad in this work suggests that it is likely to promote BCSC survival in circulation and metastasis, being a possible player of therapeutic resistance of breast cancer patients.

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P45 In vivo model of Human Colorectal Carcinoma Through Tumor-Founding Stem-like Cells Dissemination: significance and use in microbiota studies and therapeutic strategies

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Introduction: Obtaining a virtually unlimited expansion of clinically-relevant human tumor-initiating colorectal carcinomas (CRCs) cells and of circulating and metastatic cells might have considerable therapeutic implications for the study - in vitro and in vivo - of the biology of CRCs and for the evaluation of drug efficacy.

Experimental: Here we show that human CRCs do contain a minor subset of cells bearing the defining features of somatic stem cells and the ability to establish, expand and perpetuate these tumors. These tumor-initiating CRC stem-like cells (CCSCs) can establish faithful orthotopic phenocopies of the original disease, which contain cells that undergo epithelial-to-mesenchymal transition and spread into the circulatory system. While in the vascular bed, these cells retain stemness, thus qualifying as circulating CCSCs (cCCSCs). This is followed by the establishment of lesions in distant organs, which also contain resident metastatic CCSCs (mCCSCs).

Results: We describe a standardized model that recapitulates human CRC features, not captured by previous models, including the histology at the implanted site, the generation of spontaneous metastases in a progressive fashion, the metabolic features and the gut microbiome profile.

Conclusions: Here we provide a useful platform for studies of the molecular effectors that underlie CRC initiation and progression and of the biological drivers of metabolic alterations in colon carcinogenesis that is suitable for the discovery of reliable stage- specific biomarkers and the refinement of new patient-tailored therapies for the cure of deadly metastatic CRC.

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P46 Warburg Effect Inversion: Adiposity shifts central primary metabolism in MCF-7 breast cancer cells

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Introduction: Obesity is a complex disorder and a trigger to many diseases like Diabetes mellitus (DM) and breast cancer (BrCa), both leading causes of morbidity and mortality worldwide. Abnormal glucose metabolism termed 'the Warburg effect' (1) in cancer cell is closely associated with malignant phenotypes and promote the aggressiveness of several cancers, including BrCa (2).
In this study, we evaluated the BrCa cell metabolism in normoglycemia, hyperglycemia and in an obesity-mimicking condition in order to clarify potential linking mechanisms.

Experimental: MCF-7 cells were exposed to low and high glucose levels, and to 3T3-L1 adipocyte conditioned medium, thus mimicking adiposity. Cell viability, migration, proliferation, cytotoxicity and cell death assays were performed. Hormonal and lipid profile were also characterized by biochemical assays and primary metabolism was determined by NMR-based metabolomics.

Results: Our results show an increased aggressiveness in the obesity condition with an altered energy/lipid metabolism. In the experimental obesity-mimicking status, lipids and amino acids were expended while glucose was produced by tumor cells from lactate.

Conclusions: Overall, this experimentally obesity-mimicking condition not only revealed an increased tumor proliferation and aggressiveness but also disclosed a new mechanism of cancer metabolism, the 'Warburg effect inversion' characterized by a shift on tumor cells metabolism that contrasts to 'the Warburg effect'.

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P47 Targeting mitochondrial reactive oxygen species (mtROS) impairs breast cancer cell migration without affecting the cytotoxic effects of standard therapies

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P48 Interplay Between Adipose-And-Breast Tumor Tissue Fatty Acid Profiles In The Tumor Microenvironment : A Possible Contributor To Chemoresistance

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Introduction: Obesity is a major health concern among breast cancer (BC) patients, since identified as a casual factor in BC treatment resistance [1]. The latter is understudied and motivates futher investigation. Evidence on the role of fatty acids (FAs) in BC treatment resistance are lacking [2], and we therefore hypothesize that diet-induced obesity (DIO) induces inflammation altering lipid metabolism in mammary adipose tissue, and promotes chemotherapeutic treatment resistance.

Experimental: A DIO animal model was established by feeding C57BL6 mice a high fat diet for 12 weeks. After developing the DIO phenotype, breast tumour xenographs were induced in the fourth mammary gland with E0771 triple negative BC cells. Once tumours were palpable, mice received either vehicle treatment or doxorubicin treatment (12 mg/kg). Plasma Inflammatory markers, FAs profiles and lipid metabolism markers were quantified in mammary adipose and tumour tissue.

Results: DIO significantly decreased doxorubicin treatment efficacy in breast cancer tumours ($p<0.0001$). DIO also significantly increased plasma leptin ($p=0.025$) and resistin levels ($p=0.046$) and increased NFκB protein expression in mammary fat. Furthermore, DIO supressed lipogenesis (decreased SCD-1) and lipolysis (decreased HSL) in mammary adipose tissue of doxorubicin treated mice. Conversely, increased lipogenesis (increased SCD-1) and lipolysis (increased ATGL) was found in tumour tissue, leading to significant changes in FAs composition of both tissues.

Conclusions: Under obesogenic conditions BC cells suppress the storage of FAs in mammary adipose thereby increasing free fatty acid availability, and induces inflammation. This mechanism increases FAs storage in tumour tissue and thereby promotes treatment resistance.

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P50 A role for the glycerophosphodiesterase EDI3 in choline metabolism in HER2+ breast cancer

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Abnormal choline metabolism, e.g., elevated phosphocholine and total choline have been reported in breast cancer [1]. A key protein in choline metabolism is the glycerophosphodiesterase EDI3 (GPCPD1; GDE5; GDPD6), which hydrolyzes glycerophosphocholine to choline and glycerol-3-phosphate [2]. High EDI3 in endometrial and ovarian cancers was found to be associated with metastasis and worse survival, and subsequent in vitro analyses identified EDI3 as relevant for migration, attachment and spreading [2, 3]. Silencing EDI3 altered choline, as well as phospholipid metabolites, including phosphatidic acid and lysophosphatidic acid [2]. However, despite reports of deregulated choline metabolism in breast cancer, nothing is known about EDI3 in this cancer type.

EDI3 was investigated in breast cancer patients using publicly-available transcriptomics datasets and tissue microarrays. Expression and activity of EDI3 was also characterized in a panel of breast cancer cell lines of various molecular subtypes. Finally, the effect of inhibiting EDI3 either alone or in combination with chemotherapeutic agents was examined in select cell lines.

EDI3 expression is high in HER2+/ER- breast tumors. In agreement, HER2+/ER- cells have the highest expression and activity of EDI3, as well a distinctive pattern of choline metabolite levels compared to the other subtypes, which is altered by EDI3 silencing. Pharmacological inhibition and siRNA silencing of HER2 leads to decreased EDI3 expression, suggesting that EDI3 may be regulated via HER2 signaling. In addition, silencing EDI3 decreases cell viability over time, and sensitizes cells to lapatinib and trastuzumab. Ongoing work aims to understand EDI3 regulation in the context of HER2 signaling, and whether inhibiting EDI3 enhances trastuzumab/lapatinib-induced death in vivo.

EDI3 is elevated in HER2+/ER- breast cancer. Better understanding of choline metabolism in this specific cancer type may provide potential targets for treatment.

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P51 Acridine orange as an imaging and therapeutic tool for the treatment of aggressive carcinomas

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Introduction: Carcinomas are the most common type of malignant cancers, and comprise 80–90% cancer types. Although early tumor diagnosis and improved multimodality therapy have helped to reduce patient mortality, novel and more selective therapeutic approaches are needed. Intratumoral acidosis and highly acid lysosomes are common features of aggressive carcinomas [1], and could be used both for tumor imaging and targeting. Here, we propose the use of acridine orange (AO), a fluorescent cationic molecule which rapidly concentrates within lysosomes and other acidic organelles of tumor and normal cells, to target the acidic microenvironment of bone metastasis (BM) from carcinomas. Indeed, the photo- or radio-activation of AO (PDT-AO and RDT-AO, respectively) leads to highly cell cytotoxicity through the induction of lysosome-dependent cell death [2].

Experimental: Differentiated osteoclasts (OCs) and breast carcinoma cell lines (MDA-MB-231) were used as in vitro models of BM-associated cells. Cellular uptake of AO and lysosomal pH were confirmed by confocal microscopy. In vitro viability of OCs and MDA-MB-231 after PDT-AO treatment was evaluated by counting TRAcP-positive multinucleated cells and by the Alamar Blue test, respectively. The therapeutic effect of RDT-AO treatment (single dose of 5 mg/kg AO and 5 Gy irradiation) was assessed in an in vivo mouse model of BM from breast carcinoma.

Results: RDT-AO in vivo treatment significantly reduced the osteolytic area. In vitro PDT-AO was cytotoxic specifically for breast cancer cells, whereas did not affect OC viability. As a confirmation of the in vitro assay, treatment with RDT-AO was not effective in impairing OC viability. A weak lysosome stability in carcinoma cells was identified as the mechanisms underlying the efficacy of PDT-AO in bone metastasis.
Therefore, targeting lysosomal membrane stability represents a novel approach for the induction of cancer-specific cell death.

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P52 Disruption of cancer cell metabolism using targeted drug delivery systems

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Lung cancer is one of the most fatal cancers worldwide[1]. Resistance to conventional therapies remains a hindrance for patient's treatment. Therefore, the development of more effective anti-cancer drugs is imperative. Solid tumors exhibit a hyperglycolytic phenotype, leading to an enhanced lactate production and, consequently, its extrusion to the tumor microenvironment[2]. Previous published results revealed CD147$^{-/-}$ reduced lactate export in lung cancer cells and sensitized them to phenformin, a mitochondrial inhibitor, leading to a drastic decrease in cell growth in vitro and tumor growth in vivo[3]. In this study, we envision the development of CD147-targeting liposomes (LUVs) carrying phenformin and the evaluation of their efficacy to eliminate lung cancer cells. We evaluated the therapeutic effect of both phenformin and anti-CD147 antibody as well as the efficacy of LUVs carrying phenformin on A549 and H292 2D and 3D-cell growth and proliferation, and on cancer cell metabolism, migration and invasion. Our data revealed that phenformin decreased 2D and 3D-cancer cell growth. The antibody against CD147 reduced cell migration and invasion. Importantly, CD147-targeting LUVs carrying phenformin were internalized by cancer cells and impaired 2D and 3D-cancer cell growth and proliferation. Overall, these results provided convincing evidences that anti-CD147 LUVs carrying phenformin could compromise lung cancer cell behavior.

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P53 The effect of tryptophan-derived AhR ligands on melanoma cell proliferation, migration and cell death

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Introduction: The aryl hydrocarbon receptor (AhR) plays a crucial role in several physiological and pathological processes in skin, including detoxification, cellular homeostasis, skin pigmentation and skin immunity but it is also strongly associated with skin cancer induction and progression. Tryptophan-derived AhR ligands are synthesized from tryptophan via enzymatic pathway (L-kynurenine, kynurenic acid) or as a stable photoproduct 6-formylindolo[3,2-b]carbazole (FICZ) and may directly and indirectly influence melanocytes and melanoma cells.
The aim of this study was to evaluate the effect of tryptophan-derived AhR ligands: L-kynurenine, kynurenic acid and FICZ on melanoma cell proliferation, migration and induction of cell death.

Experimental: The study was conducted on human adult primary epithelial melanocytes and melanoma A375 cells. The effect of L-kynurenine, kynurenic acid and FICZ on the proliferation and cell viability of melanocytes and melanoma cells was assessed by BrdU and LDH method, respectively. Tumor Cell Transendothelial Migration Assay was applied to study the effect of tryptophan-derived AhR ligands on invasiveness of A375 melanoma cells. Induction of apoptosis and necrosis was assessed by Cell Death Detection ELISA.

Results: L-kynurenine and FICZ inhibited proliferation and DNA synthesis in melanoma cells, whereas tryptophan-derived AhR ligands did not affect proliferation and cell viability of melanocytes. Only L-kynurenine in the highest concentration (5 mM) decreased DNA synthesis in melanocytes. L-kynurenine, kynurenic acid and FICZ had no effect on the migration of A375 cells. Moreover, tryptophan-derived AhR ligands induced cell death in melanoma cells.

Conclusions: Tryptophan-derived AhR ligands, L-kynurenine, kynurenic acid and FICZ, may affect proliferation and cell death of melanoma cells in concentrations well-tolerated by melanocytes.

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P54 Metabolic adaptation in colorectal cancer: heme export is required for the down-modulation of the tricarboxylic acid cycle

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Heme is crucial for oxidative metabolism, being a key component of the electron transport chain complexes. Moreover, heme biosynthesis, by consuming succynil-CoA, is a tricarboxylic acid (TCA) cycle cataplerotic pathway and one of the major cellular iron-consuming processes, competing with the biogenesis of iron-sulfur (Fe-S) clusters. Therefore, alterations of heme homeostasis are expected to impact at several levels on metabolic adaptation of cancer cells, a key process for tumor cell growth, dissemination, immune escape and establishment of drug resistance. Here we investigated whether the control of intracellular heme amount by the cell surface heme exporter Feline Leukemia Virus subgroup C Receptor 1a (FLVCR1a) could affect heme production and, consequently, key metabolic processes relying on heme synthesis.

FLVCR1a was down-modulated in colorectal cancer cells by RNA-interference. Heme synthesis and metabolic parameters were assessed both at steady state conditions and in time-course tracing experiments.

The data show that FLVCR1 is overexpressed in cancer and that increased heme export is required in cancer to sustain heme biosynthesis. Our data indicate that, paradoxically, the down-modulation of oxidative metabolism depends on enhanced heme biosynthesis. Inhibited heme biosynthesis in FLVCR1a-silenced cells results in enhanced tricarboxylic acid (TCA) cycle flux, TCA cycle anaplerosis and oxidative phosphorylation, associated to increased activity of Fe-S clusters containing enzymes. The metabolic alterations upon suppression of heme biosynthesis-export lead to mitochondrial dysfunction and impaired cancer cell survival/proliferation.

This work identifies an unexpected role for the heme biosynthesis/export axis in the modulation of oxidative metabolism and point out the down-modulation, rather the enhancement, of heme biosynthesis to interfere with metabolic adaptation of cancer.

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P55 Acidosis drives EMT and cancer cell invasiveness through fatty acid metabolism reprogramming.

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Introduction: During cancer progression, tumor cells must adapt to survive and proliferate under low pO_2 but also low pH. We previously reported that cancer cells chronically exposed to acidosis (pH 6.5) exhibit a dysregulated fatty acid (FA) metabolism with the concomitance of FA oxidation (FAO) and synthesis (FAS). In parallel, we observed that pH6.5-adapted cancer cells exhibited an elongated and flat, mesenchymal-like appearance (vs. parental cells). This led us to suspect that acidosis could promote EMT and cancer cell invasiveness by altering metabolism.

Experimental procedures and Results: Using modified Boyden chambers, we first found that pH6.5-adapted cancer cells exhibited an increased motility and invasiveness potential. We next documented the expression of mesenchymal markers together with the loss of various epithelial markers in acid-adapted cancer cells of different tissue origins. Furthermore, we observed an accumulation of lipid droplets (LDs) in acid-adapted cancer cells, as detected by electron microscopy, Oil Red O staining and BODIPY 493/503. A CD36-dependent increase in FA uptake was found to support both FAO and neutral lipid storage into LDs. Perilipin 2 (a coat protein for LDs) and DGAT1 (an enzyme that supports triglyceride (TG) formation) were found to be highly expressed in acid-adapted cells. Importantly, we also showed that LDs not only act as energy stores but also support anoikis resistance, via the action of the ATGL enzyme that hydrolyzes TG to produce FA. Pharmacological inhibition or genetic silencing of any player of the signaling cascade (i.e. CD36, DGAT1, perilipin 2, ATGL) inhibited invasion capacities of acid-adapted cancer cells.

Conclusions: Our work provides new insights on the link between microenvironmental acidosis, fatty acid metabolism and tumor progression. This study also opens new therapeutic perspectives to oppose metastatic dissemination by targeting associated metabolic changes.

P56 Metabolic rewiring is associated with the pro-metastatic effect of anti-angiogenesis therapy in ovarian cancer

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Introduction: The angiogenesis inhibitor bevacizumab (Bev) added to platinum/taxane-based chemotherapy is becoming the standard-of-care offered to patients with ovarian adenocarcinoma. Despite a significant benefit in prolonging Progression Free Survival, patients will eventually relapse with a widespread peritoneal disease.
Our aim is to study the metabolic reprogramming associated with Bev-driven metastasis dissemination in ovarian cancer models.

Experimental: Bev was administered in maintenance regimen to female nude mice bearing OC-PDXs (ovarian cancer-patients derived xenografts). The treatment effect on malignant behaviour (abdominal effusion and metastases) and survival was evaluated in 11 OC-PDXs. The energy metabolism changes were investigated by assessing glycolysis-related genes, mitochondrial mass and Krebs cycle intermediates.

Results: Bev prolonged the life span (30-140 ILS%) of all OC-PDXs bearing mice. As it occurs in clinical settings, despite the unquestionable benefit of Bev, in some OC-PDX it was associated with a greater diffusion of the disease.
In those OC-PDXs (N=3) where the Bev benefit was accompanied by the increase of abdominal metastases, we found that Bev induced a rewiring of the energy metabolism toward a more oxidative phenotype. Lactate dehydrogenase (LDHA), lactate (MCT4) and glucose (GLUT1 and GLUT3) transporters were downregulated while the number of mitochondria was higher.
Accordingly, as demonstrated by ^{13}C-glucose tracing experiments analysis in vivo, the levels of Krebs Cycle intermediate metabolites, such as citrate, fumarate and malate increased.

Conclusions: We suggest that the metabolic energy rewiring imposed by Bev enables tumours to escape Bev therapy, thus facilitating tumour dissemination. Targeting the adaptive changes (e.g. impairing cancer cells mitochondrial respiration) possibly will help counteracting Bev-promoted malignant progression.

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P58 Downstream Targets of Proto-oncogene Activation Suggest Necessary Steps for Metabolic Reprogramming in Cancer

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Introduction: A proto-oncogene is a gene whose overexpression or activation alone leads to oncogenesis. Thus, downstream effects of proto-oncogene activation can shed light on the earliest molecular events in oncogenesis, unobscured and unperturbed by the ubiquitous mutations present in progressed macroscopic tumors.

Experimental: We assume the existence of a network of molecular interactions of proteins and genes under physiological conditions. The amount of information one can infer about one gene given the full knowledge of another gene, i.e., mutual information between genes, can be defined using statistical mechanics and information theory [1]. We posit that dysregulation of a proto-oncogene impacts other genes in proportion to their mutual information. We rank all metabolic genes according to their mutual information with 61 known proto-oncogenes. Consistently highly ranked genes are metabolic targets common to all proto-oncogenes, and can be considered necessary for metabolic reprogramming in cancer.

Results: The highest-ranked genes are LIS1, GAPDH, and V-ATPase. LIS1 is a known hematopoietic cell fate determinant and is necessary for oncogenesis. GAPDH is a key glycolytic enzyme. V-ATPase is an active vacuolar proton pump whose function is to extract protons from the cytoplasm. A significant increase of V-ATPase activity would increase pH_i and deplete ATP stores, both directly and indirectly via its effect on the mitochondrial .

Conclusions: We find, by strictly bioinformatic means, three metabolic targets just downstream of all proto-oncogenes. Targeted dysregulation of these genes has implications for recognized hallmarks of cancer: cell division, aerobic glycolysis, and cytosolic alkalinization.

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P59 Indomethacin affects polyamines metabolism in lung cancer cell lines: a KRAS-mutation associated feature?

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Introduction: Lung cancer is a leading cause of death worldwide. In cancer metabolism, polyamines are fundamental molecules for proliferation of various tumor types, including lung cancer. In colon cancer cells, indomethacin increases the abundance of spermidine/spermine-N^1-acetyltransferase (SSAT), a key enzyme for polyamines catabolism. Due that polyamine-dependent growth has been linked to the presence of KRAS mutations in colon cancer, the aim of this study was to compare the effect of indomethacin over SSAT expresion and polyamine metabolism in lung cancer cells that carry or lacks the KRAS mutation.

Experimental: A549 (KRAS mutant) and H1299 (KRAS wt) cells were cultured and exposed to indomethacin. SSAT gene expression was evaluated by real-time qPCR and protein levels were measured by western blot. Metabolic status of the cells was evaluated by GC/MS metabolomic analysis.

Results: Indomethacin increased SSAT gene expression and protein levels in H1299 and A549 cell lines. However, only in A549 cells, indomethacin significantly reduced the levels of putrescine and spermidine. Also in A549 cells, the metabolic features upstream of the polyamine pathway (ornithine and methionine) were increased, and the increase of ornithine correlated with the increase of several metabolites involved in the urea cycle.

Conclusions: Our result suggest that the metabolic impairment induced by indomethacin could be associated to the KRAS-mutation in lung cancer cells, giving information for potential new personalized alternatives for KRAS-mutated lung cancer patients.

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P60 Inducing cancer indolence by targeting mitochondrial Complex I is potentiated by blocking macrophage-mediated adaptive responses

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Introduction: Targeting respiratory complex I (CI) shows promising results as an anti-cancer strategy [1], but in some cases the data on the efficacy of CI inhibition in cancer are still conflicting [2]. The aim of this work was to identify adaptive responses triggered upon targeting CI in vivo.

Experimental: We created CI-deficient models by genetic ablation of the crucial CI assembly subunit NDUFS3 in osteosarcoma and colorectal cancer cells, which were then grown in nude mice as subcutaneous and pseudo-orthotopic xenografts. Tumorigenic potential, angiogenesis, hypoxic signalling and cellular componentes of tumor microenvironment (TME) were analyzed.

Results: The lack of CI prevented hypoxic adaptation and reduced tumorigenic potential of both types of aggressive cancers. Despite the lower tumorigenic potential, CI-deficient tumors continued to progress, suggesting they engage alternative mechanisms to ensure survival. The analysis of TME identified the abundance of tumor associated macrophages (TAMs) as a hallmark of CI-deficient tumors, suggesting they might rely on TAMs to promote survival. To prove this hypothesis, we used clodronate to deplete macrophages during CI-deficient tumor development and showed that such treatment significantly decreased tumor growth. Moreover, simultaneous administration of metformin and PLX-3397, a specific inhibitor of colony stimulating factor 1-dependent macrophage infiltration, significantly increased the efficacy of both PLX-3397 and metformin alone.

Conclusions: Targeting TAMs may be a promising approach to potentiate the effects of CI inhibitors in a combinatorial regimen to guarantee a synergistic effect in cancer therapy.

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P61 Dual-targeted pharmacological intervention against polyamine metabolism in non-small cell lung cancer (NSCLC) cells

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Introduction: Polyamines are essential molecules increased in non-small cell lung cancer (NSCLC) [1]. Ornithine decarboxylase (ODC) and S-Adenosylmethionine decarboxylase (AMD1) are the rate-limiting steps for polyamine biosynthesis, whereas polyamine oxidase (PAOX) recovers polyamines acetylated by Spermidine/Spermine Acetylase (SAT1, the catabolic polyamines enzyme) [2]. In this context, the combination of inhibitors of biosynthetic enzymes with stimulators of SAT1 would decrease intracellular levels of polyamines, supporting conventional therapies against NSCLC.

Experimental: To compare polyamine metabolism in different NSCLC cell lines, we used western blotting to measure levels of ODC, AMD1 and PAOX and GC/MS to study the metabolic status of cells. The effect of combined inhibition of polyamine metabolism was evaluated using DFMO, SAM486 and MDL72527 (inhibitors of ODC, AMD1 and PAOX, respectively) [2] and stimulating SAT1 with indomethacin [3]. MTT reduction was used as viability test and combinations were analyzed using the Loewe additivity method.

Results: NSCLC showed a different protein pattern expression and polyamine metabolic profile. This protein expression correlates with the resistance to the above-mentioned inhibitors. In KRAS-mutated NSCLC, which have increased ODC levels, indomethacin showed synergistic effect with MDL72527, whereas in those cells that AMD1 was overexpressed, indomethacin showed synergistic effect with SAM486.

Conclusions: The induction of polyamine catabolism by indomethacin enhances the effect of polyamine synthesis inhibitors such as MDL72527 and SAM486 in NSCLC. However, this effect varies depending on the basal metabolic fingerprint of each cell type.

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P62 The Lactate Receptor HCAR1 (GPR81) is required for cancer cell Survival, Migration and Proliferation

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Introduction: HCAR1 is an autocrine GPCR sensor of lactate normally expressed rather selectively in adipose tissue. However, surprisingly HCAR1 is highly expressed in solid tumors derived from many different tissues, for example in 94% of breast cancers and in pancreatic carcinomas (1). HCAR1 has been shown to be required for the metabolic reprogramming - including upregulation of lactate, monocarboxylate transporter (MCT) - which allows cancer cell survival in medium mimicking the harsh microenvironment of solid tumors (1). Knock-down of HCAR1 dramatically decreases growth and metastasis formation in xenograft cancer models in vivo (1). However, no HCAR1 antagonist has previously been described.

Experimental and Results: For studying the role of HCAR1 in metabolism in general, we have through structure-based, computational chemistry-guided means discovered novel synthetic antagonists selective for HCAR1 vs. HCAR2. The most potent (IC50 = 70 nM) and efficacious of the first-tier HCAR1 antagonist, TM00154 (2), reduced cell viability by 85% in MCF-7 cells, which express HCAR1, but not in e.g. LoVo cells, which do not express HCAR1. A structurally close low potency analog of TM00154 had no effect on cell viability. TM00154 inhibited MCF-7 cell migration and inhibited cell invasion in Matrigel by 30-40% at 24 hours when it did not yet affect cell survival. Currently the HCAR1 antagonists are being tested in respect of effects in 3D cancer spheroids and in primary patient-derived cancer cell cultures and the molecular mechanisms behind the HCAR1-mediated metabolic reprogramming of cancer cells is being analyzed to be presented at the ISCaM meeting.

Conclusion: the metabolic reprogramming of cancer cells required for tumor cell survival is dependent upon expression and autocrine signaling of the lactate receptor HCAR1 in the cancer cells and targeting of HCAR1 with antagonist could have therapeutic potential in solid tumors.

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P63 Metabolic reprogramming in vemurafenib-resistant melanoma

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Introduction: B-RAF is one of the major oncogenes involved in the tumorigenesis of melanoma, supported in part by aerobic glycolysis. B-RAF inhibitors brought a breakthrough in the treatment of melanoma, however, the vast majority of tumors develop resistance to this drug (1-3). So, the aim of the present work is to evaluate the metabolic alterations associated with resistance to vemurafenibe.

Experimental: The expression of metabolism-related proteins (MCT1, MCT4, GLUT1, HKII, PDK1, CAIX, ASCT2, GLS and GLUD1/2) was evaluated in 9 paired human melanoma samples (pre- and post-treatment with vemurafenib) using immunohistochemistry. These samples are currently being analyzed using the nCounter® Vantage 3D™ RNA Cancer Metabolism Panel (NanoString). Protein expression was also evaluated in 4 vemurafenib-resistant cell lines (and correspondent parental cell lines: A375, SK-MEL-19, SK-MEL-28 and WM164) while one of the vemurafenib-resistant cell lines (and correspondent parental cell line SK-MEL-19) was also analyzed using NanoString.

Results: In patient samples, a decrease in the expression of CAIX and MCT1 was observed after treatment with vemurafenibe. Conversely, an increase in glutaminolysis-related proteins (mainly GLUD1/2) was observed in vemurafenib-resistant melanoma cell lines. Nanostring analysis of SK-MEL-19 resistant cell line showed an increase in the expression of HIF1A, PDHA1, FASN and PDK3 and a decrease in SLC7A11 and SP1, compared to the parental cell line ($p<0.0001$).

Conclusions: Vemurafenib-resistant melanomas show metabolic alterations, opening a window to overcome vemurafenib-resistance through metabolic targeting.

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P64 IL-7 either promotes or inhibits autophagy, depending on the stress context, to promote the viability of T-cell acute lymphoblastic leukemia cells

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Introduction: Autophagy is a homeostatic degradative pathway, that mitigates stress, allows cell survival, and may be a pro-tumoral mechanism. The cytokine Interleukin-7 (IL-7) partakes in leukemogenesis. In T-cell acute lymphoblastic leukemia (T-ALL), IL-7 promotes cell proliferation, survival and metabolic activation in vitro via PI3K/Akt/mTOR pathway (inhibitor of autophagy). IL-7 can also activate MEK/Erk pathway (promoter of autophagy). Our goal was to explore the impact of IL-7 on the autophagic process in T-ALL cells and elucidate its molecular mechanisms and functional consequences.

Experimental: We used both cell lines and cells from patient-derived xenografts. Small-molecule inhibitors for PI3K, mTOR, MEK1/2 and ULK1/2 were used. Regular medium, low serum or L-Asparaginase (ASNase) culture conditions were used to test IL-7-mediated autophagy regulation. We analyzed cell viability, signaling pathway activation, LC3-I/-II conversion and puncta, and autophagosome formation.

Results: IL-7 triggers both pro- (MEK/Erk) and anti- (PI3K/Akt/mTOR) autophagic signaling in T-ALL cells. In optimal culture conditions IL-7-mediated viability relies on the latter pathway. Under stress (serum starvation) IL-7 promotes autophagy in leukemia cells, and survival partially relies on autophagy and MEK/Erk activation. In a therapy-related scenario (ASNase treatment) IL-7 promotes survival of leukemic cells that is also partially dependent on autophagy activation.

Conclusions: Our results suggest that IL-7 makes use of a 'flexible strategy' to promote T-ALL cell viability by recruiting both pro- and anti-autophagic pathways, which are differentially recruited depending on the microenvironmental conditions. In addition, combination therapies against PI3K/Akt/mTOR and MEK/Erk pathways, or combining ASNase administration with autophagy inhibitors, may be relevant in the context of T-ALL.

P65 The role of fatty acids on the metabolic reprogramming of breast cancer cells

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Introduction: Metabolic pathways are frequently dysregulated in cancer. Breast cancer cells show high glycolytic rates, intense anabolic growth, glutamine addiction, alteration of metabolic enzymes and de-novo fatty acid synthesis[1]. Historically, a competitive relationship between glucose and fatty acid usage at the level of tissue metabolism was reported, with cells ultimately relying upon only one of the two energy sources[2]. Here, we investigate how this balance is maintained and contributes to tumour progression in breast cancer.

Experimental: To investigate the effects of exogenous fatty acids on cellular metabolism, we evaluated the effects of fatty acids on the activity of Pyruvate Kinase Muscle Isozyme M2 (PKM2), the limiting enzyme that catalyses the last reaction in glycolysis.

Results: The inclusion of physiological levels of fatty acids(FAs) in the growth media resulted in uptake, membrane incorporation, and storage of exogenously supplied FAs in several breast cancer cell lines. Interestingly, we observed an insulin-independent decrease in glucose uptake and significant reduction in PKM2 activity in cells cultured in FA enriched media. Utilizing a FA-pulldown assay, we observed a robust association between Hexokinase and PKM2 and medium-long chain FAs. In order to demonstrate the direct interaction between FA and PKM2, we used a chemically synthesized 18C-FA probe containing a diazirine ring and alkyne handle. We found strong and stable interactions between recombinant PKM2 and the FA probe. Importantly, our results suggest that it is the glycolytically inactive, dimeric form, of PKM2 that preferentially interacts with the FA probe. These findings implicate FAs as direct regulators of glucose uptake and catabolism.

Conclusions: Overall, these results provides an new mechanism linking the uptake of FAs to the regulation of the glycolysis in breast cancer cells.

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P66 Protein kinase WNK1 contributes to the regulation of GLUT1 expression in the plasma membrane

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P67 Tyrosine phosphorylation modulates cell surface expression of chloride cotransporters NKCC2 and KCC3

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Introduction: Cellular chloride transport has a fundamental role in cell volume regulation and membrane potential, both in normal and tumour cells (1,2). Cellular chloride entry or exit are mediated at the plasma membrane by cotransporter proteins of the solute carrier 12 family. For example, NKCC2 resorbs chloride with sodium and potassium ions at the apical membrane of epithelial cells in the kidney, whereas KCC3 releases chloride with potassium ions at the basolateral membrane. Their ion transport activity is regulated by protein phosphorylation in response to signaling pathways. An additional regulatory mechanism concerns the amount of cotransporter molecules inserted into the plasma membrane.

Experimental: Co-transporter constructs were transfected into HEK293 cells and the activity of SYK kinase modulated by incubation with SYK inhibitors or by co-transfection with siRNAs, kinase-dead, or constitutively active SYK mutants. Co-transporter abundance in the plasma membrane was analyzed by biotinylation of cell surface proteins.

Results: Here we describe that tyrosine phosphorylation of NKCC2 and KCC3 regulates their plasma membrane expression levels. We identified that spleen tyrosine kinase (SYK) phosphorylates a specific N-terminal tyrosine residue in each cotransporter. Experimental depletion of endogenous SYK or pharmacological inhibition of its kinase activity increased the abundance of NKCC2 at the plasma membrane of human embryonic kidney cells. In contrast, overexpression of a constitutively active SYK mutant decreased NKCC2 membrane abundance. Intriguingly, the same experimental approaches revealed the opposite effect on KCC3 abundance at the plasma membrane, compatible with the known antagonistic roles of NKCC and KCC cotransporters in cell volume regulation.

Conclusions: We identified a novel pathway modulating the cell surface expression of NKCC2 and KCC3 and show that this same pathway has opposite functional outcomes for these two cotransporters. The findings have several biomedical implications considering the role of these cotransporters in regulating blood pressure and cell volume.

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P68 NADPH oxidases and Warburg effect in Chronic Myeloid Leukemia: a history of metabolic addiction

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Introduction: Chronic myeloid leukaemia (CML) is a haematological neoplasm characterized by the expression of the constitutively active kinase BCR-ABL. This kinase triggers ROS production via NADPH oxidases (NOX) (1)including cancer. However, a moderate amount of reactive oxygen species (ROS and induces the Warburg effect as part of its transforming activity (2). However, as far as we know, it is unknown if the metabolic switch induced by BCR-ABL is dependent on ROS production by NOX. Therefore, we wondered whether NOXs could be involved in the metabolic adaptation of CML cells (3).

Experimental: We analyze the effect of NOX inhibition on key metabolic parameters: glucose uptake, lactate dehydrogenase (LDH) activity, Krebs cycle intermediates, mitochondrial respiration and glycolysis (Seahorse, Agilent), ATP/ADP, NADH/NAD+ and NADPH/NADP+ levels. Inhibition of NOX was done both chemically (DPI) and by shRNA against $p22^{phox}$ or Nox2 subunits in K562 cell line.

Results: Silencing of Nox2 is able to promote an increase in LDH activity together with a rise in glucose uptake. In addition, we were able to detect that these cells were more dependent on mitochondrial metabolism. This fact was marked by an increase in basal and maximum oxygen consumption rate, ATP production and spare respiratory capacity.

Conclusions: Considering that Nox2 silencing enhanced mitochondrial metabolism, we hypothesize that NOX activation by BCR-ABL might be important for inducing the Warburg effect. We are currently analyzing how Nox2 can regulate mitochondrial metabolism.

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P69 Obesity/type 2 diabetes mellitus biomarkers induce changes in proliferation, viability, migration, apoptosis and nutrient transport in breast cancer cells

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Introduction: Obesity and type 2 diabetes mellitus (T2DM), which are becoming increasingly prevalent worldwide, associate with increased incidence and mortality from many cancers[1], including breast cancer. The mechanisms involved in this relation remain poorly understood. So, we investigated the effect of obesity/T2DM biomarkers (hyperglycemia, hyperinsulinemia, hyperleptinemia, and increased levels of inflammation and oxidative stress) upon nutrient transport and breast cancer progression.

Experimental: Breast cancer cells (MCF7 and MDA-MB-231), were exposed to glucose (15-20 mM), TBH (0.5-2.5 μM), insulin (1-50 nM), leptin (10-500 ng/ml) and TNF-α or INF-γ (1-100 ng/ml) for 24 h, and cell proliferation rates, culture growth, cell viability, apoptosis rates, migration and ^{3}H-deoxy-D-glucose (^{3}H-DG) and ^{3}H-glutamine (^{3}H-Gln) uptake was quantified.

Results: In both cell lines, insulin and leptin increased cell proliferation rates and migratory capacity and both compounds induced apoptosis in MCF-7 cells, although their effect on cell viability (decreased by insulin and increased by leptin) was distinct. Their positive effect on cell proliferation rates and migratory capacity correlated with an increase in ^{3}H-DG uptake.

In both cell lines, TNF-α decreased the cell proliferation rates, culture growth and the % of viable cells, but increased the migratory capacity and apoptosis index (MCF-7 cells only). INF-γ decreased the cell proliferation rates in both cell lines, and decreased the migratory capacity, increased the % of viable cells and increased the apoptosis index in MCF-7 cells only.

TBH decreased the % viable cells and the apoptosis index in both cell lines, but its effect on proliferation rates and migration was distinct in MCF-7 (decrease) and MDA-MB-231 (increase) cells. Interestingly, these effects correlated with its effect on ^{3}H-Gln uptake.

Conclusions: Obesity/T2DM biomarkers induce changes in nutrient transport that can contribute to cancer progression.

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P70 Selective pro-apoptotic and antimigratory effect of polyphenol complex catechin:lysine 1:2 in cancer cell lines

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Introduction: Cancer is a major cause of death in both developed and developing countries. Polyphenols, abundantly found in plants, possess many anticarcinogenic properties, including inhibition of cancer cell proliferation, tumor growth, angiogenesis, metastasis and inflammation, as well as pro-apoptotic effects[1]. Our study aimed to investigate the effects of a complex of (+)-catechin with 2 lysines (Cat:Lys) on cancer and non-cancer cells.

Experimental: For this, the in vitro effect of Cat:Lys on the viability, growth, proliferation, apoptosis, nutrient uptake and migration of breast, pancreas and colon cancer and non-cancer cell lines was evaluated.

Results: Cat:Lys exerted antiproliferative and cytotoxic and effects in all breast, pancreas and colon cell lines tested, but with a much less marked effect in non-cancer cell lines. It nevertheless interfered with nutrient (^{3}H-deoxy-D-glucose and ^{3}H-lactate) uptake and with lactate production in both cancer and non-cancer cell lines. Cat:Lys was found to possess a selective antimigratory effects in breast, pancreas and colon cancer cell lines compared to non-cancer cell lines. Cat:Lys also exerted pro-apoptotic effects in all the cancer cell lines that we tested, but not in non-cancer breast and pancreas cell lines. The antimigratory, but not the pro-apoptotic, effects of Cat:Lys were found to be mediated by JAK2/STAT3 and Wnt pathway inhibition.

Conclusions: In conclusion, Cat:Lys is a strong candidate for the development of a new, effective anticancer agent against cancer.

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P71 PFKP mediated aerobic glycolysis in Triple Negative Breast Cancer (TNBC): a potential therapeutic target

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Introduction: Triple Negative breast cancer (TNBCs) cells demonstrate high aerobic glycolysis. Previously, we have demonstrated that of the three Phosphofructokinase (PFK) isoforms (Liver-L; Muscle-M and Platelet-P), high expression of PFKP is associated with decreased breast cancer patient survival. In the present study, we explored the role of PFKP in breast cancer progression and metastasis.

Experimental: For experiments, we utilized two TNBC cell lines (MDA-MB-231 and MDA-MB-468), having high expression of endogenous PFKP expression. siRNA/or Quercetin mediated knockdown of PFKP was carried out and breast cancer migration/ invasion was evaluated (using trans-well assay), along with lactate estimation. PFKP protein expression was also evaluated in breast cancer specimens (n=50) by Immunohistochemical analysis.

Results: Silencing of PFKP/or Quercetin treatment significantly depleted PFKP expression in TNBCs cells. PFKP siRNA/Quercetin treatment showed impaired migration and invasion of TNBC cells suggesting that PFKP regulate aerobic glycolysis in breast cancer cells thereby regulating their migration and invasion. Additionally, treatment inhibited the lactate production in TNBC cells, as compared to respective controls. Cytoplasmic accumulation of PFKP protein was observed in breast cancer patients, as compared to the benign breast tissue suggestive of the fact that PFKP is a differentially expressed gene in breast cancer, validating our on-line meta-analysis data.

Conclusions: Our study highlighted the importance of PFKP in TNBC progression, thereby providing a therapeutic window where antagonists of PFKP (i.e Quercetin) can be utilized for breast cancer therapy either alone or in combination.

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P72 Iron availability regulates cancer related skeletal muscle wasting

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P73 Prognostic value of Monocarboxylate transporter 1 overexpression in different cancer types: a systematic review

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Introduction: The cancer cell glycolytic phenotype produces high amounts of lactate that is extruded through monocarboxylate transporters (MCTs), enhancing tumour microenvironment acidification, which is associated with cancer cell aggressiveness. MCT1 activity/overexpression is associated with both lactate uptake and efflux, playing an important role in the existing metabolic symbiosis in the tumour microenvironment.
We aimed to assess the prognostic value of MCT1 overexpression in different cancer types by performing a systematic review.

Experimental: Searches were performed both on PubMed and Google Scholar on February 26th, 2019, with the following keywords: monocarboxylate transporter 1, lactate transporter, SLC16A1, cancer, survival and prognosis. Studies were included from inception, full-text articles written in English, studies with prognostic information on human cancer patients based on survival parameters and studies including MCT1 protein expression evaluation. Duplicates were excluded. Extracted data were: country of sample origin, type of study design, patient number, age, sex, cancer type, immunohistochemistry information, outcome measures and results of each study.

Results: 288 studies were identified in the initial search. The final analysis retrieved 15 studies, which were subjected to qualitative synthesis. 12 out of the 15 studies presented significant results: 92% of these studies (11 in total) reported a significant association between MCT1 overexpression and decreased survival in different cancers. However, the methodological approaches diverge among the included studies, which compromise comparisons.

Conclusions: MCT1 overexpression is associated with worse prognosis in different cancer types. Nevertheless, to support future application in clinical practice, further investigation on this topic is needed, including standardization of methodological procedures.

P74 A patient with undifferentiated pleomorphic sarcoma treated with new protocol of radiotherapy after intravenous injection of acridine orange, targeting cancer acidic autolysosomes

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Introduction: Acridine orange (AO) is proven to accumulate into acidic cancer autolysosome and kill cancer cells by apoptosis after photo or X-ray irradiation (photodynamic or radiodynamic effect). We lately reported clinical outcome of low-dose radiotherapy after intravenous injection of AO (iAOR) for 8 patients with cancer in terminal clinical stage. Results revealed that 3 out of 5 patients who completed the treatment showed a clinical or imaging-based response (60% of response rate) and that all patients did not have any complication after AO injection. In the study, patients received 5 Gy X ray radiation after 1mg/kg AO injection at a one-week interval three times over 3 weeks. Based on these results, we confirm that systemic administration of AO is not toxic in humans, and iAOR is potentially effective against cancer. Therefore, in this study we apply new protocol of iAOR with increasing total AO administration dose and total radiation dose to the patient with undifferentiated pleomorphic sarcoma (UPS) showing frequent local recurrence and lymphnode metastasis after intensive chemotherapy, surgery and radiotherapy.

Experimental: Patient who was 40 year-old woman had a UPS tumor over the left patella and treated with chemotherapy and wide resection surgery in 2009. Local recurrence and lymph node metastasis appeared at the left inguinal in 2014. Until 2018, she received frequent surgeries and intensive chemotherapy and radiotherapy, however tumor recurred at whole of the left extremity and metastasized to the inguinal and intrapelvic lymph nodes. Especially tumor at the left thgh rapidly increased and caused serious pain. In order to relieve synptom, we started iAOR therapy with new protocol (10 times treatment with 3 Gy radiation after 1mg/kg AO injection with 2 or 3 days interval) to tumor at the thigh and metastatic lymph nodes at the inguinal.

Results: Macroscopic tumor size gradually decereased at 5 times treatment and pain was relieved. At 2 months after iAOR treatment, tuomr volume decreased 27% by CT image and lymph nodes metastatic lesion disappeared by PET image.

Conclusions: New protocl of iAOR is more effective than old one, because old one did not show drastic decrese of tumor size.

P75 3-Bromopyruvate pre-treatment sensitizes MOLM13 and KG-1 cell lines to chemotherapy

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Introduction: Acute Myeloid Leukemia(AML) is the most common adult acute leukemia and it is characterized by clonal expansion of immature myeloblasts1. The most common treatment is the combination of daunorubicin(DNR) with cytarabine(AraC). Unfortunately, the relapse rate is high and the outcome is poor2. Most cancer cells present altered energetic metabolism relying on aerobic glycolysis, even under aerobic conditions ("Warburg effect"). 3-Bromopyruvate(3BP) is an alkylating agent that targets cancer cell metabolism, and has been demonstrated to be a powerful antitumor agent either in in vitro and in vivo models, but little is known about its effect in leukemia models3. Our aim was to evaluate a new therapeutic approach, in which cells are pre-treated with a 3BP non-toxic concentration followed by DNR or AraC treatment and understand the mechanism of action of 3BP pre-treatment.

Experimental: MOLM13 and KG-1 cell lines were treated with 5μM 3BP for 16h or 0.5mM 2-deoxyglucose(2DG) for 24h. After that, 105cells were cultured in 24-well plates and treated with a range of DNR or AraC concentrations for 48h. Cell viability and IC50 values were determined by the Trypan Blue assay. For metabolic characterization, we measure extracellular glucose and lactate concentrations (commercial kits, according to the manufacturer's instructions), reactive oxygen species(ROS) and mitochondrial activity by flow cytometry. The molecular probes Dihydroethidium(DHE) and red/green Mitotracker used were for ROS level determination and mitochondrial activity, respectively.

Results: 3BP pre-treatment enhanced the effect of chemotherapy drugs, decreasing cell viability and IC50 values for KG-1 and MOLM13 cell lines. After incubation with 3BP, only MOLM13 cells presented a decrease in glucose consumption, which was not reflected in the decrease of lactate levels. However, 5μM of 3BP disrupted mitochondrial activity and increased ROS levels.

Conclusions: Pre-treatment with non-toxic concentrations of 3BP sensitize AML cells to chemotherapeutic agents likely not by alteration in the glycolytic profile but by mitochondrial activity disruption.

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P77 Ketogenic diet as an auxiliary treatment for melanoma

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P78 Metabolic targeting of Neuroblastoma with "old drugs"

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Introduction: Neuroblastoma (NB) is a childhood cancer characterized by a subpopulation of high-risk patients who have still a poor outcome. Cancer cells are characterized by a high dependency on glucose. In addition, NB cells have low but still functional Oxidative phosphorylation (OXPHOS) complexes [1]. Recent studies suggest that inhibition of residual OXPHOS activity can be beneficial in cancer treatment. Since, antibiotics such as doxycycline (Dox) and tigecycline (Tig) and anti-diabetic drugs as metformin (Met) target mitochondrial energy metabolism, these compounds may have an antitumor effect on NB [1, 2]. The aim of this study was to investigate the effect on the proliferation of NB cells with different genetic background.

Experimental: The NB cell lines SH-SY5Y (non-NMYC-amplified) and SKNBE(2) (NMYC- amplified) were treated for 3 days with different concentrations of Dox, Tig and Met and cell viability was assessed by MTT. The effect of Met on tumor growth was evaluated in an intradermal xenograft model, by using SKNBE(2) cells. Met treatment (50 or 100 or 250 mg/kg/mice/day by oral gavage) was started when the tumor size reached ~100 mm3.

Results: Treatment of SH-SY5Y and SKNBE(2) with Tig (10 µM, 30 µM, 100 µM) and Dox (30 µM, 100 µM) led to a dose dependent decrease of NB cell proliferation. Furthermore, Met (10 mM, 20 mM) also reduced proliferation of both cell lines. In SKNBE(2) xenografts model the treatment of metformin (100, 250 mg/Kg) led to a delay in tumor growth. However, there was no significant difference of the tumor volume at the termination day.

Conclusions: The reduction of NB growth in vitro by treatment with antibiotics and metformin opens up new adjuvant therapies, which are urgently needed in NB treatment of high-risk patient.

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P79 Hexokinase 2 locates at mitochondria-endoplasmic reticulum contact sites of cancer cells and its displacement prompts Ca2+-dependent death

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P80 Commensal gut bacteria modulate immune surveillance and promote extra-intestinal tumor overgrowth

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P81 Anti-VEGF therapy promotes lipid accumulation and storage in ovarian cancer xenografts

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Introduction: Anti-VEGF therapy increases hypoxia and causes nutrient starvation triggering metabolic perturbations both in experimental and clinical tumors[1]. Metabolic adaptation is mainly represented by exacerbated glycolysis and lactate production. De-regulation of additional metabolic processes caused by anti-VEGF therapy has been less investigated so far[2,3].

Experimental: Mice with ovarian cancer xenografts were treated with the anti-VEGF mAb bevacizumab for 4 weeks. Tumor samples were analyzed by LC-MS and NMR-MS to measure lipid content and lipid storage was also evaluated by IF staining with the Lipid Droplets (LD) associated marker adiphophilin. Tumor sections were analyzed for expression of proteins/enzymes involved in lipid metabolism by IHC and tumor RNA was processed for transcriptome analysis. Finally, we investigated whether drugs targeting lipid metabolism could counteract lipid accumulation in tumor cells in vitro under hypoxia.

Results: By using both LC-MS and NMR-MS techniques, we found that treatment of ovarian cancer xenografts caused tumor schrinkage associated with model-specific alterations of the lipidomic profile. These alterations were accompanied by increased accumulation of LD. In bevacizumab-treated tumor, we detected by IHC increased expression of enzymes involved in fatty acid biosynthesis, including ACSS2, ACC and FAS, and reduced expression of CPT1A, a β-oxydation enzyme. Moreover, transcriptome analysis uncovered up-regulation of pathways involved in lipid metabolism. In vitro experiments showed that LD accumulation was promoted by hypoxia and lipid storage could be impaired by lipid-targeting drugs.

Conclusions: Overall, our results show that lipid accumulation is a common adaptation of tumors to VEGF blockade and suggest that this phenomenon might be counteracted by lipid metabolism drugs.

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P82 Regulation of PERK expression by FOXO3: a vulnerability of drug-resistant cancer

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Introduction: The major impediment to effective cancer therapy has been the development of drug resistance. The tumour suppressive transcription factor FOXO3 promotes cell cycle arrest, senescence and cell death, and mediates the cytotoxic and cytostatic functions of cancer therapeutics. In consequence, FOXO3 is often down-regulated as an adaptive response in cancer and particularly in chemotherapeutic drug-resistant cells.

Experimental & Results: We find that FOXO3 expression is attenuated in the drug-resistant MCF-7-EpiR and MCF-7-TaxR compared to the parental MCF-7 breast cancer cells. Using ChIP, siRNA knockdown, and overexpression assays as well as Foxo1/3/4 -/- MEFs, we establish the endoplasmic reticulum (ER)-stress defence modulator PERK (eIF2AK3) as a direct downstream transcriptional target of FOXO3. In agreement, there is also a positive correlation between FOXO3 and PERK expression at the protein and RNA levels in breast cancer patient samples. We uncover that PERK expression is downregulated but its activity constitutively elevated in the drug resistant cells. With this in mind, we exploit this adaptive response of low FOXO3 and PERK expression and high PERK activity in drug-resistant breast cancer cells and show that these drug-resistant cells are specifically sensitive to PERK inhibition. In support of this finding, we show that ectopic overexpression of FOXO3 can reduce the sensitivity of the resistant cells to the PERK inhibitor GSK2606414, while the Foxo1/3/4 -/- MEFs expressing lower levels of PERK are more sensitive to PERK inhibition compared to wild-type MEFs. PERK inhibitor-titration and -time course experiments showed that the drug-resistant cells which express lower expression and higher activity levels of PERK are more sensitive to the increasing concentrations of PERK inhibitor compared to parental MCF-7 cells.

Conclusions: Our present work thus reveals a chemotherapeutic drug-resistant cancer cell vulnerability in PERK and suggests PERK as a potential target for cancer therapy, specifically in the context of drug-resistant cancers.

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P83 Accelerated glucose metabolism is associated with less T cell infiltration in human OSCC

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Introduction: The oral squamous cell carcinoma (OSCC) belongs to the group of head and neck cancers and is the sixth most prevalent malignant disease worldwide (1). It commonly but not exclusively affects older male individuals and is associated with extensive abuse of tobacco and/or alcohol, HPV or asbestos exposure. Our aim was to characterize the metabolic phenotype as well as the immune cell status of OSCC to achieve possible new treatment strategies for OSCC.

Experimental: For this purpose we analyzed tumor tissue, mucosa and blood of 21 patients with OSCC. Flow cytometry was used to analyze tumor infiltrating immune cells (T and myeloid cells). Additionally, mRNA analyzes of lactate dehydrogenase A (LDHA), monocarboxylate transporter-1 (MCT-1) and glucose transporter-1 (GLUT-1) were performed via qPCR. Metabolic parameters, such as lactate, glucose, Lp(a), triglyzerides were analyzed in serum of patients and healthy individuals. Moreover, CD3, CD4, Foxp3 and GLUT-1 were analyzed in a tissue microarray of 229 OSCC patients.

Results: We could clearly show that OSCC demonstrates a Warburg phenotype which is in line with the findings of other groups. Tumors exhibited a strong upregulation of glycolytic markers, like LDHA, GLUT-1 and MCT-1. While lymphocytes were decreased in peripheral blood of tumor patients, higher numbers of peripheral myeloid cells could be detected. Comparing tumor tissue with corresponding healthy mucosa, we could also observe a significant decrease of intra-tumoral lymphocytes while myeloid cells were increased. Tumor-infiltrating CD4+ T cells were mainly CD25+Foxp3+. Of importance, high CD3+ infiltration in the tumor front and less GLUT-1 expression in the tumor lesions correlated with an improved recurrence-free survival in OSCC patients.

Conclusions: Enhanced glycolysis in OSCC is associated with less infiltration of anti-tumoral lymphocytes as well as an accumulation of tumor-promoting regulatory CD4+ T cells. Targeting the Warburg phenotype of tumor cells may therefore improve the efficacy of checkpoint therapy in human OSCC.

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P84 The effects of metformin on MDA-MB-231 breast cancer cells in a monolayer culture and in tumor spheroids as a function of nutrient concentrations

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Introduction: Metabolic pathways of cancer cells depend on nutrient concentrations in their micro-environment. They also differ between a standard 2D monolayer culture and 3D tumor spheroids, in which three-dimensional growth of cells better mimics a tumor. Recently, we have shown that medium renewal and glucose concentrations modulate the effects of metformin on MDA-MB-231 cells in a 2D culture. Here we compared the effect of metformin on MDA-MB-231 cells in a standard 2D culture and in 3D tumor spheroids as a function of glucose, pyruvate and glutamine concentrations. Furthermore, we examined the effects of metformin in commonly used media (DMEM, MEM and RPMI-1640) that differ mainly in the concentrations of amino acids.

Experimental: We used MTS assay and Hoechst and propidium iodide staining to determine cell viability, number and survival, respectively. We also determined the size of tumor spheroids and assessed effects of nutrients on metformin-stimulated AMP-activated protein kinase activation using Western blotting.

Results: Non-essential amino acids suppressed the effects of metformin on MDA-MB-231 cells in a 2D culture and in 3D tumor spheroids. Glutamine and pyruvate weakly diminished the effects of metformin in 2D culture. Furthermore, glucose protected tumor spheroids against metformin-induced disintegration.

Conclusions: Our results show that nutrient availability must be considered when we evaluate the effects of metformin in 2D culture and in biologically more relevant 3D tumor spheroids.

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P85 Exploiting serine hydroxymethyltransferase moonlighting function: novel perspectives to target serine metabolism in cancer cells

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Introduction: serine hydroxymethyltransferase (SHMT) converts serine to glycine to produce tetrahydrofolate-bound one-carbon units. SHMT is involved in pathways leading to purine, pyrimidines and antioxidant molecules[1]. Interestingly SHMT is often over expressed in tumors. While many chemotherapeutic agents have been developed to inhibit other folate cycle enzymes, SHMT still remains an elusive target. Considering the low efficacy in vivo of the small molecules tested in the last years, we decided to better elucidate SHMT biology to explore novel strategies[2].

Experimental: we evaluated in vitro the binding ability of SHMTs to RNA (by EMSA), and the inhibition of the enzymatic activity (spectrophotometry). We induced the expression of target sequences specifically in the mitochondria of lung cancer cells. Then we evaluated the biological effects as cell death (by annexin-V etc), production of reactive oxygen species (by DCFDC) mitochondrial metabolism (seahorse).

Results: we clarified the moonlighting function of the cytoplasmic isoform of SHMT (SHMT1) which, by binding the 5'UTR of the mitochondrial isoform (SHMT2) regulates its expression. Interestingly RNA binding to SHMT1 inhibits the serine cleavage activity directing serine into the mitochondria to provide one-carbon units as formate and antioxidants as NADPH and glutathione[3]. We also demonstrated that SHMT1 inhibition by SHMT2-5'UTR overexpression in lung cancer cells induces metabolic alterations, ROS production and cell death.

Conclusions: Our data suggest that the moonlighting function of these proteins could be explored as an alternative approach to regulate their intracellular function and effectively as a new anticancer strategy.

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P86 Exploiting the antitumor activity of the metabolic modulators 3-BP, DCA and 2DG in a lung cancer model

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Conventional chemotherapies are particularly toxic to cancer cells; however, they are responsible for significant side effects associated to treatment[1,2]. The use of more specific and effective compounds is a goal in the field of cancer research. "Warburg effect" is a cancer hallmark, consisting in a metabolic shift in energy production from oxidative phosphorylation to glycolysis, even in the presence of O2[3]. Continuous activation of glycolysis gives rise to rapid energy and lactate increase, promoting proliferation, invasion and chemotherapy resistance[4]. 3-bromopyruvate (3BP), dichloroacetate (DCA) and 2-deoxyglucose (2DG) are anti-glycolytic agents that inhibit cancer cell metabolism, depleting cellular ATP[5,6]. The sensitivity of the lung cancer cell lines A549 and NCI-H460 to these compounds was assessed and their effect on viability, metabolism, proliferation and migration was characterized.

Cell viability of compounds was assessed by the sulforhodamine B assay. The metabolism was analyzed through lactate, glucose and ATP quantification, upon exposure of the cells to the compounds. Their effects on cell proliferation and migration were determined by % BrdU incorporation and wound healing assay, respectively.

NCI-H640 was found to be the most sensitive cell line to all compounds. These differences were not due to MCT1 expression (the main 3BP transporter) and/or CD147 (its chaperone). Concerning the metabolism, a lower glucose consumption and lactate production was found, but the most notorious effect was on ATP levels, with all compounds inducing a depletion of energy in both cell lines. 3BP was the only agent that decreased in a significant way the migration, but only in NCI-H460 cells. It was also observed that DCA was the metabolic modulator that induced higher inhibition of proliferation in both cell lines.

These findings allow us to conclude that these compounds can have potential for metabolic modulation and inhibition of growth in lung tumors.

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P87 Targeting glucose metabolism and mitochondria-induced apoptosis in cancer cells: A structure-based virtual screening validation toward Hexokinase 2 inhibitors

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Introduction: Glucose is regarded as the main fuel of cancer cells and the glycolytic pathway has been demonstrated as a potential target to be explored for cancer treatment. Several enzymes involved in glycolysis are overexpressed in different types of cancer cells, namely hexokinase 2 (HK2)[1]. This enzyme is not only involved in the first and most determinant step of glycolysis and subsequently in the different branched pathways[2,3], but also in the immortalization of cancer cells. When catalytically active, HK2 is able to bind to the voltage-dependent anion channel (VDAC) in the mitochondrial outer membrane, preventing the normal pro-apoptotic signalling. HK2-VDAC disruption would promote the binding of pro-apoptotic proteins to VDAC, stimulating the enhancement of apoptosis in cancer cells[4]. In this way, the inhibition of the HK2 catalytic centre is proposed as a strategy to reduce the main source of energy to cancer cells, thus significantly decreasing cancer cell proliferation and avoiding HK2 binding to VDAC and thereby enhancing the apoptosis process.

Experimental: As an effort to find hit compounds able to interfere with the HK2 catalytic activity, a structure-based drug design strategy was implemented, leading to the virtual screening of several general databases such as DrugBank (~2000 molecules), NCI (~265 000 molecules), Chemoteca (~800 molecules) and some specific natural product derivatives databases such as Ambinter (~10 000 000 molecules) and InterBioScreen Natural Products (~84 000 molecules). The virtual screening was carried out using molecular docking calculations through Gold 5.20 software. Molecules were prepared using Molecular Operating Environment (MOE2016 0802) and then docked into the HK2 catalytic site. Biochemical validation of the above-mentioned protocol was conducted using the ADP-GloTM kinase assay, a luminescence-based approach. The compounds were tested at 10 μM.

Results: Our in silico studies have identified 2981 molecules with the potential to act as new HK2 inhibitors. Preliminary results of biochemical evaluation with 64 selected molecules are presented. Twenty-two molecules were found to inhibit? the HK2 activity more prominently or in the same range of the known inhibitor 3-bromopyruvate (3BP).

Conclusions: The experimental data support the predictions of the structure-based virtual screening procedure. All the 64 molecules tested in the kinase assay affect HK2 activity to some extent. The results demonstrate that the in silico procedure used herein can recognize bioactive molecules, ready for structural optimization and/or further testing.

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P88 Chromene derivatives: a new strategy to overcome renal cancer resistance

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Renal cell carcinoma (RCC) is the most frequent and lethal tumor of the urological system. Due to late diagnosis, metastatic RCC is very recurrent, requiring systemic treatment combined with surgery. However, the effectiveness of these treatments is poor, motivating the discovery of new therapeutic approaches. Diverse signaling pathways have been linked to altered metabolism in renal carcinogenesis and tumor progression, being current targets in RCC therapy. However, drug resistance eventually becomes problematic due to genetic and epigenetic modifications or downstream pathway activation.

In the present study, A498 cells were subjected to drug pressure with stepwise increasing dosage over time. Furthermore, inspired by in silico screening results, new chromene derivatives were synthetized and tested on rapamycin-resistant A498 cells attempting to overcome the acquired resistance and to characterize their mode of action.

After 32 weeks, cells developed drug resistance to rapamycin 7-fold higher than the IC50 value of the parental cell line, confirmed by increased expression levels of mTOR. Lactateproduction and glucose consumption is altered in rapamycin-resistant cells compared to the parental A498 cell line. These metabolic alterations seem to be mediated by transcriptional factors, including HIF-1α, -2α and c-Myc. Moreover, despite their resistance to rapamycin, cells treated with the newly synthetized chromene-based molecules preserve IC50 values in the nanomolar range, similarly to the parental cells. Finally, exploring the effect of the compounds, preliminary results show a decrease in mTOR expression by Western-blot, indicating sensitivity of resistant-cells to these chromenes.

Our study opens new perspectives for the treatment of renal cell cancer resistant to targeted therapy.

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P89 New ruthenium-based anticancer agents: uncovering the molecular targets towards colorectal cancer therapy

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Colorectal cancer (CRC) is the second cause of cancer death in Portugal[1]. CRC therapy has limited chemotherapeutic agents available. CRC with mutations in KRAS, BRAF and PIK3CA and overexpressing EGFR are resistant to available EGFR inhibitors, which constitutes a clinical relevant problem that needs to be overcome[2,3]. Ruthenium (Ru) drugs had arisen as one of the most promising metallodrugs with features that increase their specificity and selectivity toward cancer cells[4,5]. For these reasons, four new ruthenium complexes were synthesized, one taking advantage of Ru anticancer properties (PMC79) and three resulting from different targeting approaches (PMC78, LCR134 and LCR220). Here, we want to unravel the effect and mechanism of action of Ru compounds in CRC cells. For that purpose, we used two CRC-derived cell lines with different genetic background (KRAS and BRAF mutations) and a normal colon cell line, to analyze cell viability, cell cycle, cell death, MAPK-ERK and PI3K-AKT signaling pathways, actin cytoskeleton and GLUT1 expression. Our results showed that our compounds are more cytotoxicity for cancer cells, showing an IC50 at the micromolar range. Ru compounds induce apoptosis but do not interfere with cell cycle. Moreover, our compounds seem to influence differently the expression of AKT and ERK in the two CRC cell lines. PMC79 inhibited to a high extent the expression of AKT and ERK proteins in CRC cells with KRAS mutation. Ru compounds also affected F-actin polymerization and β-actin expression suggesting that actin might be a possible target for these compounds. Additionally, PMC79 upregulated the expression of GLUT1 in CRC cells with KRAS mutation suggesting that it might interfere with glucose metabolism. Moreover, the combination of PMC79 with a GLUT1 inhibitor potentiated the Ru compound effect. Overall our results showed that all compounds present promising anticancer activity in CRC cells. The effect of the Ru compounds is dependent on the genetic background of the cells, with a more pronounced effect observed on CRCs harboring KRAS mutation, what could bring new avenues in CRC therapy.

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P90 Unveiling the role of microbiome-derived short chain fatty acids in colorectal cancer metabolism

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Colorectal cancer (CRC) is one of the most commonly diagnosed cancers worldwide[1]. Besides the hereditary causes, diet has been identified as an important risk factor[2,3,4]. Specific dietary patterns can modulate the bacterial species that constitute the intestine microbiome, which play different roles in human health, such as protection against pathogens, immune system maturation, degradation of toxic substances, digestion of complex carbohydrates and production of short-chain fatty acids (SCFAs).

Propionibacteria are responsible for the production of SCFAs, namely acetate, propionate and butyrate[5]. Our group already showed that acetate inhibits CRC cell proliferation, induces apoptosis, promotes lysosomal membrane permeabilization, increases CRC cell glycolytic phenotype and regulates its own uptake by increasing the expression of monocarboxylate transporters (MCTs)[6,7]. The protective effects of butyrate against CRC is linked to inhibition of cell differentiation, promotion of cell-cycle arrest and apoptosis, inhibition of the inflammatory response and modulation of histone acetylation[8,9,10,11]. Propionate triggers anti-survival mechanisms, being also associated with the inhibition of inflammatory responses and modulation of histone acetylation[12,13].

With this work, we aim to assess the effects of different SCFAs, alone and in combination, in the regulation of the metabolism in normal colon cells and in CRC-derived cell lines with different genetic backgrounds. We decided to test four different ratios of SCFAs: one mimicking the physiological conditions and three others representing different dietary profiles (dairy rich and poor diet) and a dysbiosis scenario.

Our preliminary results showed that at least acetate increases the expression levels of MCT-1 and MCT-4, maintaining the uptake of SCFAs. To the best of our knowledge, this is the first report addressing the role of SCFAs in the modulation of the energetic metabolism in CRC, which might help in the discovery of new approaches in prevention/therapy of CRC.

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P91 Benzo[a]phenoxazines derivatives as potential novel agents for colorectal cancer therapy

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P92 Oncogenic KRAS mutations may modulate the pro-apoptotic protein BAX at the mitochondria

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P93 Impact of L- and D- Kynurenine on T cell function

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Introduction: Metabolic conditions in the tumor microenvironment (TME) often form a barrier for effective immune response to tumors. Tumors might show increased tryptophan metabolism, leading to accumulation of kynurenines (kyn). Kyn are ligands of the aryl hydrocarbon receptor (AhR) which has been linked with immunosuppressive effects. Several mechanisms of immunosuppression may be mediated by the kynurenine pathway including depletion of tryptophan, direct immunosuppression of kyn, and activity of kyn-bound AhR. In order to shed some light on the impact of kynurenines on T cells, we investigated the effect of both isomers L- and D- kynurenine on the phenotype, metabolism and function of different T cell subtypes.

Experimental: Human CD4+, CD4+25- and CD8+ T cells were isolated from peripheral blood using either FACS or MACS technology. The cells where then stimulated with anti-CD3/28 T cell expander beads for 3 (Tconv) or 4 (iTregs) days in the presence or absence of different concentrations of L-and D- kynurenine. Afterwards, cells were counted, proliferation, apoptosis and activation markers were measured by flow cytometry. The supernatants were harvested and cytokines were measured by ELISA.

Results: We could clearly show that both CD4+ and CD8+ T cells undergo apoptosis when treated with high concentrations (1 mM) of both kynurenine isomers. In lower concentrations (500 µM), CD8+ T cells appear to be more affected than CD4+ T cells. The cell proliferation, but not T cell activation, seem to be impaired in the same concentrations for both T cell subtypes. Analysis of culture supernatants revealed that INF-□ secretion of CD8+ T cells and to a lesser extent of CD4+ T cells, was suppressed through kyn.

Analysis of naïve CD4+ T cells (CD4+25- cells) demonstrated that both isomers had a strong impact on cell proliferation with addition of 1mM of kynurenine. However, in lower concentrations, (500 µM), only D-kynurenine had a significant impact on cell proliferation. Adding an increased amount of AB serum to the culture was able to partially rescue the cells, but only if treated with the L- isomer.

Conclusions: Our data demonstrate that kynurenines have an impact on T cell phenotype, and that different T cell subtypes show different susceptibilities to kynurenine. Although the majority of studies make use of L-kynurenine, we demonstrate a strong effect of the D-isomer, especially in naïve CD4+ T cells. Culture conditions also prove to be important since supplementing the media with a higher percentage of AB serum seem to protect naïve cells from the effects of L- kynurenine.

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P94 Evaluation of antitumor activity of AZD3965 using in vivo models: a systematic review

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Introduction: Monocarboxylate transporter 1 (MCT1) mediates proton-lactate coupled transport in cancer cells. MCT1 inhibition by AZD3965 induces cancer cell metabolic changes and compromises tumor growth in vivo. However, there are no secondary studies on its antitumor activity, justifying the assessment of the strength of the current preclinical evidence. Our aim was to evaluate AZD3965 antitumor activity using in vivo models, compared to control vehicle or other intervention.

Experimental: Article search was performed using Cochrane Database of Systematic Reviews, PROSPERO, NICE Evidence, Ovid Online, TRIP, PubMed and Google Scholar. In vivo primary studies with comparative groups on AZD3965 antitumor activity, using AZD3965 and a comparative control vehicle or other intervention were selected in duplicate. For data analysis, AZD3965 antitumor activity was compared per tumor type.

Results: AZD3965 treatment alone or combined with radiotherapy increases tumor lactate. AZD3965 induces tumor growth inhibition (except in Diffuse Large B-Cell Lymphoma (DLBCL) or Colorectal Carcinoma (CRC)), that is enhanced after co-administration of doxorubicin, rituximab, radiotherapy, simvastatin or JNJ-605. AZD3965 reduces tumor burden, displays limited antitumor efficacy, lower glutamate/succinate, does not change tumor pH and glycolysis intermediates, improves bioenergetics and Fluoro-deoxyglucose uptake and induces transient pyruvate changes. Responses were maintained after cessation of AZD3965 treatment alone or with rituximab. One study states that engraftment progressed after AZD3965 treatment alone or with BAY 87-2243. Combination of AZD3965 with simvastatin increases time to reach 4-fold the initial tumor volume. Either AZD3965 alone or with BAY 87-2243, simvastatin or rituximab and 50mg/kg of AZD3965 (unlike 100mg/kg) with doxorubicin were well tolerated.

Conclusions: We summarized the positive antitumor activity of AZD3965 and combination therapy except in DLBCL and CRC and its good tolerability. However, the available preclinical evidence does not seem strong enough and probably more studies should be conducted to support clinical trials.

P95 Integration of lipid metabolism with Krebs cycle in acute myeloid leukemia

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Introduction: Acute myeloid leukemia (AML) is the most common type of acute leukemia in adults, yet has the lowest survival rate. Such prognostic has not changed over the past decades as well as the therapeutic strategy that has also not been significantly modified over the last 40 years. Although the current therapeutic regimes result in a positive response in young patients, intensified therapy is highly toxic, has limited application and poor outcomes among old individuals, the most affected population, resulting in high mortality. Mounting evidence suggests that metabolic reprogramming contributes to cancer development, and in fact, chemotherapies targeting metabolism have demonstrated to be effective cancer treatments. Our previous results suggested that the functioning and implications of autophagy and the metabolic pathways in leukemia are dependent on the AML subtype disclosing that inhibition of AKT is a promising therapeutical target in some scenarios. Furthermore, it was also suggested that targeting the "de novo" fatty acid synthesis might be advantageous in AML context.

Experimental: Therefore, the goal of this project was the characterization of the intracellular fluctuations of different species of metabolites and to evaluate the lipids content of three heterogeneous AML cell lines, namely the KG-1, NB-4 and HL-60.

Results: Data obtained revealed that KG-1 cells exhibited preferential OXPHOS metabolism with increased accumulation of neutral lipids and citrate, in comparison with NB-4 and HL-60 cells, which in turn presented a similar metabolic profile.

Conclusions: These results are also correlated with isocitrate dehydrogenases activity highlighting an exclusive metabolic profile for each tested AML cells and its impact on determination of the anti-leukemia efficacy and on personalized combinatory therapy with conventional and targeted agents.

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P96 Skin remodelling and damage prevention by and antioxidant-rich juice

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Introduction: Skin cancer is the most prevalent cancer in Brazil and has high incidence rates worldwide. Ultraviolet radiation (UV) from sunlight is able to induce skin damage characterized by alterations of extracellular matrix, DNA damage, cell lesion and apoptosis, which may culminate in cancer development. Food antioxidants, as flavonoids, carotenoids and antioxidant vitamins are able to neutralize reactive oxygen species, modulate cell growth and apoptosis, which may counteract the degenerative actions of UV sunlight. Therefore, the aim of this study was to evaluate the likely protective effects of an antioxidant-rich juice (ARJ, composed by orange, carrot, honey, tomato extract, avocado, ginger and camu-camu manufactured by Metabolics®), in rats submitted to skin damage by UV exposure.

Experimental: 20 male Wistar rats were divided in 4 groups: control group (C) – animals received water and were not exposed to UV light; UV damage group (UVD) – received water and were exposed to UV radiation; ARJ group (ARJ) – received the antioxidant juice ad libitum and were not exposed to UV radiation; and ARJ damage group (ARJ+UV) – received the antioxidant-rich juice and were exposed to UV radiation. The ARJ was offered during 21 days to ARJ and ARJ+UV groups. Rats from UVD and ARJ+UV were exposed to UV light for 2 non consecutive days, during five hours each day, after 15 days of juice supplementation. On the 22nd day, rats were killed by decapitation and epithelium samples from the dorsal skin removed, fixed in bouin and embedded in paraffin. The sections were stained with hematoxylin and eosin or mallory and picrosirius red.

Results: Increased collagen deposition was observed in UV groups, yet ARJ treatment prevented collagen degradation and extracellular matrix remodelling.

Conclusions: UV radiation significantly caused sunburn of superficial epithelial cells of C. The ARJ, which is rich in vitamin C and other antioxidants, was able to protect against acute skin damage.

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P97 Effect of leucine supplementation on the reversal of muscle mass loss in cancer patients and the elderly subjects

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Introduction: The purpose of the study was to verify the effect of leucine (LEU) supplementation, an essential amino acid, in the gain of muscle mass and/or in the reversal of muscle loss in patients with cancer or in elderly subjects.

Experimental: Patients with cancer (C) (n=15) and elderly volunteers (non-cancer - NC) (n=13) were enrolled in a double-blind, randomized, placebo-controlled study and were divided into 2 subgroups: LEU or placebo (PLA), 6g/day for 28 days. The supplementation effects were analyzed and compared (intra and intergroup). The skeletal muscle mass index (SMI) was evaluated by dual-energy X-ray absorptiometry (DEXA); functional evaluations were performed using gait speed (GS) and handgrip strength (HG). The classification of muscle loss and cut-off points followed the EWGSOP2 protocol. Statistical analysis included 3-way ANOVA with Tukey or Bonferroni correction to compare the groups, the effects of supplementation versus placebo and pre and post-supplementation; 2-way ANOVA or Mann-Whitney were used to compare the variations between pre and post-supplementation in the groups. The significance level adopted was $p<0.05$.

Results: The SMI variation between pre and post-intervention was greater in NC group than C group when supplemented with LEU (P=0.046). Handgrip Strength was higher in the C group when compared to NC group, regardless of the supplementation type or pre and post-intervention (p=0.004).

Conclusions: The Supplementation protocol failed to elicit major differences in the groups studied.

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