Program:

Registration/coffee 9.30

Chair – Gyorgy Szabadkai

9.50-10.00	**Gyorgy Szabadkai** (UCL) Introduction: Do mitochondria have a unique role in cancer?
10.00-10.50:	***Opening Guest lecture***: **Adrian Harris**, *(Oxford, MRC Weatherall Institute of Molecular Medicine):* Adaptation of hypoxia and mitochondrial metabolism in breast cancer
10:50-11:20:	**Dimitrios Anastasiou** (MRC National Institute for Medical Research): Roles of metabolic reprogramming in cancer

Coffee 11.20-11.40. ……..

11.40-11.50	**Abcam:** David Bruce
11.50-12.20	**Margaret Ashcroft** (Division of Medicine, UCL): HIFs and mitochondria harness tumour hypoxia
12.20-12.35	**Ali Tavassoli** (University of Southampton): A cyclic peptide inhibitor of C-terminal binding protein dimerization links metabolism with mitotic fidelity in breast cancer cells.
12.35-12.50	**Michelangelo Campanella** *(Royal Veterinary College, UCL) T*SPO and VDAC1 regulate a ROS-mediated pro-survival pathway by *i*nhibiting Mitochondrial Quality Control

Lunch 12.50-1.40 ……..

Chair – Margaret Ashcroft

1.40-2.10	**Ivan Gout** (Department of Structural and Molecular Biology, UCL): Regulation of cell growth and energy metabolism by ribosomal S6 kinases
2.10-2.40:	**Sarah-Anne Martin** (Barts Cancer Institute, QMUL): Targeting the mitochondria for the treatment of MLH1-deficient disease
2.40-2.55	**Zhi Yao** (Department of Cell and Developmental Biology, UCL) PGC-1beta mediates adaptive chemoresistance associated with mitochondrial DNA mutations
2.55-3.10	**Luisa Iommarini** (University of Bologna) Different mtDNA mutations modify tumor progression in dependence of the degree of respiratory complex I impairment

Tea – 3.10-3.30

3.30-3.45	**Ajit S. Divakaruni** (Department of Pharmacology, UCSD): Inhibition of the mitochondrial pyruvate carrier potentiates metabolic flexibility
3.45-4.00	**Susana Ros** (Cancer Research UK London Research Institute) The Role of the glycolytic enzyme PFKFB4 in Growth and Survival of Cancer Cells
4.00-4.50	***Closing Guest lecture*** **Christian Frezza** (Cambridge, MRC Cancer Unit): Cancer metabolomics and mitochondria

To be followed by posters and a reception with drinks and nibbles.

Poster Index

Poster 17 Barbara Pernaute (Imperial College London)
Embryonic stem cells become primed for cell death upon the onset of differentiation

Poster 18 Sukaina Rashid (Queen Mary University of London)
Targeting the mitochondria for the treatment of MLH1-deficient disease

Poster 19 Sarah Rodrigues Moreira (Laboratoire de Recherche sur la RÃ©paration et la Transcription dans les Cellules Souches, France.)
Very Low Doses of Radiation Lead to Long Term Defects of Hematopoietic Stem Cells Functions

Poster 20 Margarida Sancho (Imperial College London)
Competitive elimination of defective cells during early mouse embryo development

Poster 21 David Selwood (University College London)
Mitochondrial targeting of cyclosporin analogues

Poster 22 Vicky Sherwood (University of East Anglia)
Wnt Signalling Reprogrammes Cancer Cell Metabolism

Poster 23 Renaud VATRINET (University di Bologna)
An anti-tumorigenic effect ascribed to severe respiratory complex I mutations

Poster 24 Ralf Zwacka (University of Essex)
Tumour suppressing and tumour progressing activities of MnS

Abstracts - Oral

Abstract 1

Adaptation of hypoxia and mitochondrial metabolism in breast cancer in response to Avastin and metformin

Adrian Harris

Weatherall Institute of Molecular Medicine, Oxford

Hypoxia is recognised to induce a multigene programme mainly via HIF1a and also HIF2a transcription factors. Bioinformatics analysis of multiple gene array data sets in breast cancer showed a core hypoxia response programme of approximately 90 genes associated with poor outcome independently of other factors. This core response was significantly over-expressed in triple receptor negative cancers. Additionally, microRNAs associated with hypoxia were shown to give additional worse prognosis associations (mir-210). mir-210 targeted the mitochondrial iron chaperone responsible for regulation of key enzymes in the Krebs cycle and showed an adaptive response to hypoxia involving switching off the mitochondrial metabolism.

To assess in human breast cancer the hypoxia transcriptome we conducted gene microarray studies before and after 2 weeks of bevacizumab 15mg/kg single dose before neoadjuvant chemotherapy. Extensive gene induction occurred, including many components of the HIF pathway, but also glycogen metabolism and lipid metabolism.

We investigated these further in xenograft models to see which of the adaption pathways may be most important for survival under hypoxic conditions. Reactivating mitochondria under hypoxic conditions induced by angiogenesis also showed additional anti-cancer benefits and is the basis now for a new phase I study in our department.Surprisingly, induction of glycogen and lipid storage occurred and this was essential for survival on reoxygenation and for protection against free radical damage, which greatly increased when either pathway was inhibited.

We investigated, by bioinformatic approaches, the expression of 133 key enzymes in metabolism, showed that they were strongly associated with different subtypes of breast cancer, which may help in selection of patients for future intervention studies.

To further define the hypoxia transcription, we conducted RNA sequencing of MCF7 cells in normoxia and mild hypoxia. This revealed marked induction of many long non-coding RNAs, suppression of all transfer RNAs and induction of novel antisense RNAs.

Overall, therefore, although anti-angiogenic therapy alone is now withdrawn from clinical utility in breast cancer, the massive induction of hypoxic microenvironment and synergy with many other therapeutics, suggests that as new approach using induced essentiality should be reassessed in breast cancer.

This work was funded by Cancer Research UK, Breast Cancer Research Foundation, Inspire 2 Live, Oxford Biomedical Research Centre.

Notes:

Abstract 2

Functions of metabolic reprogramming in cancer

Dimitrios Anastasiou

MRC National Institute for Medical Research, London

Solid tumours are thought to develop over a long period of time the later stages of which are associated with increased proliferation. One function of altered metabolism in cancer is to support this hyperproliferative phenotype by promoting the biosynthesis of macromolecules. Concomitantly, though, analyses of genetic lesions that occur early during cancer development indicate that metabolic reprogramming may precede the need for increased proliferation, suggesting additional functions of metabolism in tumorigenesis.

We have shown that the M2 isoform of the glycolytic enzyme pyruvate kinase (PKM2) mediates metabolic changes that help cancer cells withstand oxidative stress. PKM2 is expressed in a large spectrum of solid tumours and in some normal tissues. Unlike its highly homologous alternatively spliced variant PKM1, PKM2 can be inhibited by reactive oxygen species (ROS) produced in mitochondria to promote utilisation of glucose for antioxidant responses. Abrogation of this homeostatic mechanism by expression of a ROS-insensitive mutant or small molecule activators of PKM2 increase sensitivity of cells to oxidative stress and inhibit xenograft tumour growth in mice. These studies suggest that metabolic reprogramming in cancer, in this case through expression of a ROS-responsive glycolytic enzyme isoform, can function as a defence mechanism against stress. The implications of these observations for the design of strategies to interfere with glycolytic metabolism in cancer will be discussed.

Notes:

Abstract 3

HIFs and mitochondria harness tumour hypoxia.

M Ashcroft

University College London, London

Research over the last decade has greatly increased our understanding of how cells sense and respond to changes in cellular oxygen levels. The hypoxia inducible factor (HIF) family of bHLH-PAS transcription factors are central components of the cellular oxygen-sensing machinery in mammalian cells. Previous studies have highlighted an important role for mitochondria in regulating HIF and the hypoxic response. However, the molecular mechanisms that interface mitochondria with the HIF/hypoxia response remain to be unravelled. We have identified and characterized a family of mitochondrial proteins that regulate cellular oxygen consumption rate and metabolism, and provide a critical role in hypoxia signalling and tumour progression (1). These and other novel links between the HIF/hypoxia pathway and mitochondria (2) provide new molecular insights into harnessing hypoxia and metabolic adaptive responses within the tumour microenvironment.

(1) Yang J, Staples O, Thomas LW, Briston T, Robson M, Poon E, SimÃµes ML, El-Emir E, Buffa FM, Ahmed A, Annear NP, Shukla D, Pedley BR, Maxwell PH, Harris AL, Ashcroft M (2012). Human CHCHD4 mitochondrial proteins regulate cellular oxygen consumption rate and metabolism and provide a critical role in hypoxia signaling and tumor progression. J Clin Invest. 122(2):600-11.

(2) Briston T, Yang J, Ashcroft M (2011). HIF-1a localization with mitochondria: a new role for an old favorite? Cell Cycle. 10(23):4170-1.

Notes:

Abstract 4

A cyclic peptide inhibitor of C-terminal binding protein dimerization links metabolism with mitotic fidelity in breast cancer cells.

Ali Tavassoli

University of Southampton

Identification of direct modulators of transcription factor protein-protein interactions is a key challenge for ligand discovery that promises to significantly advance current approaches to cancer therapy. Here, we report an inhibitor of NADH-dependent dimerization of the C-terminal binding protein (CtBP) transcriptional repressor, identified by screening genetically encoded cyclic peptide libraries of up to 64 million members. CtBP dimers form the core of transcription complexes associated with epigenetic regulation of multiple genes that control many characteristics of cancer cells, including proliferation, survival and migration. CtBP monomers also have distinct and critical cellular function, thus current experimental tools that deplete all forms of a targeted protein (e.g. siRNA) do not allow the cellular consequences of this metabolically regulated transcription factor to be deciphered. The most potent inhibitor from our screen (cyclo-SGWTVVRMY) is demonstrated to disrupt CtBP dimerization in vitro and in cells. This compound is used as a chemical tool to establish that the NADH-dependent dimerization of CtBPs regulates the maintenance of mitotic fidelity in cancer cells. Treatment of highly glycolytic breast cancer cell lines with the identified inhibitor significantly reduced their mitotic fidelity, proliferation and colony forming potential, whereas the compound does not affect mitotic fidelity of cells with lower glycolytic flux. This work not only links the altered metabolic state of transformed cells to a key determinant of the tumor cell phenotype, but the uncovered compound also serves as the starting point for the development of potential therapeutic agents that target tumors by disrupting the CtBP chromatin-modifying complex.

Notes:

Abstract 5

TSPO and VDAC1 regulate a ROS-mediated pro-survival pathway by inhibiting Mitochondrial Quality Control

MICHELANGELO CAMPANELLA

The Royal Veterinary College, London

The 18kDa Translocator Protein (herein TSPO) localises on the Outer Mitochondrial Membrane (OMM) and partakes in the synthesis of cholesterol, overexpressed in mammalian tumours its ligands are used to map their progression and severity.

We show that its ratio of expression with the Voltage Dependent Anion Channel 1 (VDAC1), to which it binds, its critical to dictate an accumulation of defective mitochondria that leads to apoptosis resistance, and increased proliferative capacity. This is consequence of mitochondrial autophagy (or mitophagy) inhibition, which occurs downstream of the PINK1/Parkin pathway via the prevention of essential ubiquitination of OMM proteins.

Independent of cholesterol synthesis, the mitophagy modulation by TSPO is instead dependent on VDAC1, via which it downregulates mitochondrial Ca2+ buffering capacity and ATP generation but promotes an accumulation of the Reactive oxygen species (ROS) that negatively affect the Parkin mediated ubiquitination of OMM proteins. This leads to an accumulation of dysfunctional mitochondria that are resistant to chemotherapeutic agents as well as an upregulation of ROSâ€“dependent Protein Kinases and Transcription Factors.

This set of data identifies TSPO as a novel element in the regulation of mitochondrial quality control by autophagy, and demonstrates the importance of the expression ratio between TSPO and VDAC1 for cell metabolism and potential contributing element toward genesis and progression of tumours.

Key words: TSPO, Cancer, Mitochondria, Mitophagy, Ca2+, ROS, PINK1/Parkin Pathway, Ubiquitin

Notes:

Abstract 6

Regulation of ribosomal S6 kinases in normal and cancer cells

Ivan Gout

University College London, London

Ribosomal protein S6 kinase (S6K) is a member of the AGC family of Ser/Thr kinases which also includes PKA, PKB (Akt), PKCs etc. Biochemical and genetic studies in cell-based and animal models have provided evidence that S6K is a principal player in the regulation of cell growth, size and energy metabolism. Two major signal transduction pathways, phosphatidylinositide 3-kinases (PI3K) and mammalian target of rapamycin (mTOR), coordinate the activity of S6Ks in response to extracellular and intracellular stimuli, such as growth factors, mitogens, metabolites and nutrients. In an activate state, S6Ks translocate to discrete cellular compartments/multienzyme complexes, where they interact with and phosphorylate diverse substrates implicated in the regulation of translation, RNA processing, cytoskeletal rearrangement, cell growth and survival. A growing body of evidence links S6K signalling to various human pathologies, including diabetes, ageing and cancer.

In mammalian cells, there are two isoforms of S6K, termed S6K1 and S6K2. The activity and subcellular localization of S6Ks are regulated by multiple S/T phosphorylations in response to diverse extracellular stimuli. Furthermore, acetylation and ubiquitination have been implicated in regulating the function of S6Ks. We have recently uncovered a novel mode of S6K activation, mediated by specific interaction with DNA. Data on molecular mechanisms underlying this regulatory event and its involvement in coordinating transcription, proliferation and cell survival, and in mediating chemoresistance to anti-cancer drugs will be presented.

Notes:

Abstract 7

Targeting the mitochondria for the treatment of MLH1-deficient disease

Sukaina Rashid, Gemma Bridge & Sarah A Martin

Queen Mary University of London, London

The DNA Mismatch repair (MMR) pathway is responsible for the repair of base-base mismatches and insertion/deletion loops that arise during DNA replication. MMR deficiency is currently estimated to be present in 15-17% of colorectal cancer cases and 30% of endometrial cancers. With the aim of identifying compounds that cause selectively lethality upon MLH1 deficiency, we screened MLH1-deficient and proficient cells, with a chemical library of 1120 compounds. Strikingly, upon analysis, a number of the hit compounds have been shown to target mitochondrial function. Our previous work has shown that MLH1 can localize to the mitochondria and silencing of the mitochondrial, genes POLG and PINK1 are synthetically lethal with MLH1 deficiency. We demonstrate that loss of MLH1 is also associated with reduced mtDNA content, reduction in Complex I activity and reduced oxidative consumption rate, suggesting that mitochondrial function is deregulated upon loss of MLH1.

Notes:

Abstract 8

PGC-1beta mediates adaptive chemoresistance associated with mitochondrial DNA mutations

Zhi Yao

University College London, London

Primary mitochondrial dysfunction because of mutations of mitochondrial DNA (mtDNA) has been recently recognized as a major culprit in ageing and age-related degenerative diseases, and is often associated with loss of cellular proliferation, senescence and cell death. Nonsynonymous mutations of mtDNA are frequently found in cancer cells and may have a causal role in the development of resistance to genotoxic stress induced by common chemotherapeutic agents, such as cis-diammine- dichloroplatinum(II) (cisplatin, CDDP). However, little is known about how these mutations arise and the associated mechanisms leading to chemoresistance. Here, we show that the development of adaptive chemoresistance in the A549 non-small-cell lung cancer cell line to CDDP is associated with the hetero- to homoplasmic shift of a nonsynonymous mutation in MT-ND2, encoding the mitochondrial Complex-I subunit ND2. The mutation resulted in a 50% reduction of the NADH:ubiquinone oxidoreductase activity of the complex. This reduction of complex-I activity triggers compensatory increase of respiratory chain complexes biogenesis as well as increased extracellular acidification rate indicating an increased glycolysis in the chemoresistant A549 cells. The compensatory mitochondrial biogenesis was most likely mediated by the nuclear co-activators peroxisome proliferator-activated receptor gamma co-activator-1alpha (PGC-1alpha) and PGC-1beta, both of which were significantly upregulated in the CDDP-resistant cells. Importantly, both transient and stable silencing of PGC-1beta re-established the sensitivity of these cells to CDDP-induced apoptosis. Remarkably, the PGC-1beta-mediated CDDP resistance was independent of the mitochondrial effects of the co-activator. Altogether, our results suggest that partial respiratory chain defects because of mtDNA mutations can lead to compensatory upregulation of nuclear transcriptional co-regulators, in turn mediating resistance to genotoxic stress.

Notes:

Abstract 9

Different mtDNA mutations modify tumor progression in dependence of the degree of respiratory complex I impairment

Luisa Iommarini

University di Bologna, Bologna, Italy

Mitochondrial DNA mutations are currently investigated as modifying factors impinging on tumor growth and aggressiveness, having been found in virtually all cancer types and most commonly affecting genes encoding mitochondrial complex I subunits. However, it is still unclear whether they exert a pro- or anti-tumorigenic effect. We here analyzed the impact of three homoplasmic mtDNA mutations (m.3460G>A/MT-ND1, m.3571insC/MT-ND1 and m.3243A>G/MT-TL1) on osteosarcoma progression, chosen since they induce different degrees of oxidative phosphorylation impairment. In fact, the m.3460G>A/MT-ND1 mutation caused only a reduction of complex I activity, whereas the m.3571insC/MT-ND1 and the m.3243A>G/MT-TL1 mutations induced a severe structural and functional complex I alteration. As a consequence, this severe complex I dysfunction determined an energetic defect associated with a compensatory increase in glycolytic metabolism and AMP-activated protein kinase activation. Osteosarcoma cells carrying such marked complex I impairment displayed a reduced tumorigenic potential both in vitro and in vivo, when compared to cells with mild complex I dysfunction, suggesting that mtDNA mutations may display diverse impact on tumorigenic potential depending on the type and severity of the resulting oxidative phosphorylation dysfunction. The modulation of tumor growth was independent from reactive oxygen species production but correlated with hypoxia inducible factor 1a stabilization, indicating that structural and functional integrity of complex I and oxidative phosphorylation are required for hypoxic adaptation and tumor progression.

Notes:

Abstract 10

Inhibition of the mitochondrial pyruvate carrier potentiates metabolic flexibility

Ajit Divakaruni

La Jolla, CA, United States of America

The mitochondrial pyruvate carrier (MPC) transports cytoplasmic pyruvate into the matrix, and is therefore a crucial branch point in cellular energetics. The compartmentalization of pyruvate not only balances oxidative phosphorylation and glycolysis, but also regulates catabolism, anabolism, and redox status. Previous work in our laboratory established that inhibition of mitochondrial pyruvate uptake can acutely (< 2 h.) regulate cellular glucose handling, increasing glucose uptake at the plasma membrane and activating AMPK.

In an effort to develop the MPC as a therapeutic target for cancer and other diseases rooted in aberrant glucose metabolism, we studied the consequence of chronic MPC inhibition using a combination of bioenergetics and 13C metabolic flux analysis. Remarkably, stable genetic repression of the pyruvate carrier in cultured cells (C2C12 myoblasts and A549 epithelial cells) continued to proliferate without a demonstrable growth defect, maintaining ATP-linked respiration and oxidative metabolism. Uncoupler-stimulated respiration in C2C12 myoblasts showed increased sensitivity to both the glutaminase inhibitor BPTES and etomoxir upon knockdown of either obligatory MPC paralog, potentially indicative of increased fatty acid and glutamine oxidation in these cells. The results were confirmed by metabolic flux analysis, which showed that cells maintained lipogenesis and TCA cycle flux upon MPC knockdown through increased reliance on glutamine. The pattern of glutamine oxidation revealed an increase in reductive carboxylation as well as a robust increase in glutaminolysis. Isotopic labeling studies revealed fatty acid oxidation was increased as well. Taken together, the data suggest that reductions in MPC activity rewire central carbon metabolism to potentiate â€˜metabolic flexibilityâ€™ â€“ the oxidation of alternative substrates. Importantly, these results could be reproduced with the specific MPC inhibtor UK5099, showing the MPC is indeed a druggable therapeutic target that can change the cellular pattern of substrate utilization.

Notes:

Abstract 11

The Role of the glycolytic enzyme PFKFB4 in Growth and Survival of Cancer Cells

Susana Ros

Cancer Research UK London Research Institute, London

Cancer cells preferentially use glycolysis instead of oxidative phosphorylation as a main source of energy production. This provides cancer cells with a growth advantage under challenging conditions. However, cancer cells also need to adapt their metabolism to support the increased rate of macromolecule synthesis for rapid cell growth and proliferation. We devised an unbiased screening strategy utilizing siRNA mediated gene knockdown to identify metabolic enzymes selectively required for survival of prostate cancer cells.

Among the genes selectively required for prostate cancer cell survival was the glycolytic enzyme PFKFB4, an isoform of phosphofructokinase 2 (PFK2). PFK2 is a bifunctional enzyme that regulates the levels of fructose 2,6-biphosphate, a strong allosteric activator of the most important regulatory enzyme of glycolysis phosphofructokinase 1 (PFK1). PFK2 could then turn the distribution of metabolites between glycolysis and the pentose phosphate pathway. We found that silencing of PFKFB4 increased fructose-2,6-bisphosphate levels, lowered NADPH and reduced glutathione levels resulting in enhanced oxidative stress and cancer cell death. Furthermore, silencing of PFKFB4 also blocked tumour growth in vivo, and even caused regression of the initial tumour burden. Interestingly, PFKFB4 is expressed at higher levels in metastatic prostate cancer compared to primary tumors. Indeed, the requirement of PFKFB4 for cell survival extends beyond prostate cancer. We are currently elucidating further the molecular mechanisms that promote cancer cell dependency on PFKFB4.

Understanding the contribution of this key regulatory component to the metabolic reprogramming in cancer cells is crucial for the development of novel strategies that could perturb their metabolic balance and selectively target cancer.

Notes:

Abstract 12

The metabolic transformation of Fumarate Hydratase deficient cells

Christian Frezza

University of Cambridge, Cambridge

Loss of function of the tricarboxylic acid (TCA) cycle enzyme Fumarate Hydratase (FH) leads to hereditary leiomyomatosis and renal cell cancer (HLRCC), a hereditary cancer syndrome characterized by a highly malignant form of renal cell cancer. Although the link between loss of FH and tumorigenesis has been extensively studied, no mechanism to explain the ability of FH-deficient cells to survive without a functional TCA cycle has been proposed. We hypothesised that in order to compensate for the lack of a critical enzyme of the TCA cycle, FH-deficient cells must undergo a complex metabolic rewiring. Importantly, we reasoned that by blocking those alternative pathways required by these cells to overcome the loss of FH, we could selectively kill mutant cells, sparing the FH-proficient cells. In our laboratory, we are using of a combination of metabolomics, biochemistry and computational biology to investigate the metabolic adaptations present in FH-deficient cells. We have recently found that fumarate accumulation leads to chronic GSH depletion and oxidative stress. Of note, cells rearrange their metabolism to increase antioxidant metabolic strategies and become dependent on exogenous cysteine to maintain GSH biosynthesis. We have also demonstrated that persistent oxidative stress is mechanistically associated to the emergence of senescence, which we propose is a tumour suppressive mechanism that might explain why Fh1-deficient animals do not develop overt kidney carcinomas but only benign renal cysts. Together, these findings reveal that cells undergo several metabolic changes in order to compensate for the loss of Fh1 and that these adaptations are required to allow fumarate to accumulate and exert its oncogenic activity.

Notes:

Abstracts – Posters

Poster 1

A specific mitochondrial biogenesis pattern underlies tumour development

Robert Bentham, Cathy Quin, Kevin Bryson and Gyorgy Szabadkai

University College London

Cellular transformation and proliferation are fostered by radical rearrangement of cellular metabolism, resulting in unique expression patterns along a series of metabolic pathways in cancer. Mitochondria are central to many of these pathways, suggesting that the transcriptome underlying mitochondrial biogenesis will also follow a distinctive pattern in tumour tissues. Nonetheless, few studies have addressed this question so far. In order to explore the expression pattern of nuclear encoded mitochondrial genes in cancer, we applied machine-learning algorithms to predict gene correlations between mitochondrial genes and oncogenic pathways. In silico analysis of all MitoCarta genes [1] in a dataset of ~1000 cancer cell lines and tissues (CCLE [2]) revealed a striking pattern of mitochondrial gene expression, which was specific to cancer cells. These results provide a novel framework to understand the role of mitochondria in cancer.

1] D. J. Pagliarini, S. E. Calvo, B. Chang, S. A. Sheth, S. B. Vafai, S.-E. Ong, G. A. Walford, C. Sugiana, A. Boneh, W. K. Chen, et al., â€œA mitochondrial protein compendium elucidates complex I disease biology,â€ Cell, vol. 134, no. 1, pp. 112â€“123, 2008.

[2] J. Barretina, G. Caponigro, N. Stransky, K. Venkatesan, A. A. Margolin, S. Kim, C. J. Wilson, J. Leha Â´r, G. V. Kryukov, D. Sonkin, et al., â€œThe cancer cell line encyclopedia enables predictive modelling of anticancer drug sensitivity,â€ Nature, vol. 483, no. 7391, pp. 603â€“607, 2012.

Notes:

Poster 2

Role of mitochondrial sulfide metabolism in colorectal cancer cell lines

Jennifer Bolsee, Guido Bommer

University Catholique de Louvain, Bruxelles, Belgium

Background:

It is increasingly recognized that hydrogen sulfide H2S plays a role as cellular messenger, likely by sulfhydration of thiol groups (i.e. the generation of hydropersulfides) (1). However, it has been questioned whether H2S can directly modify thiol groups or whether an intermediary step is required. Some data suggest that sulfane sulfur (e.g. in the form of polysulfides) might be the active intermediary (2). In the present project, we wanted to explore whether the first step in H2S detoxification (mediated by mitochondrial protein sulfide quinone reductase like, SQRDL) might be required to generate sulfane sulfur that is subsequently used for the sulfhydration of target proteins.

Methodology:

We generated derivatives of the HT29 colorectal cancer cell line with inducible expression of shRNAs targeting SQRDL. Sulfide detoxification into thiosulfate was assessed by HPLC. Signal transduction downstream of TNFalpha was assessed by qPCR of target genes.

Results:

SQRDL knockdown in HT29 cells reduced H2S-induced thiosulfate production by up to 80%. This was not associated with changes in signal transduction downstream of TNFalpha.

References:

1. Paul et al in Nat Rev Mol Cell Biol. 2012 Jul 1113(8):499-507

2. Greiner et al in Antioxid Redox Signal. 2013 November 20 19(15): 1749â€“1765.

Notes:

Poster 3

Pathophysiology and treatment of mitochondrial dysfunction in ME/CFS â€“ a clinical audit

Norman E Booth [1], Sarah Myhill [2], John McLaren-Howard [3]

[1] *Department of Physics, University of Oxford, and Oxfordshire ME Group for Action, Oxford UK*

[2] *Sarah Myhill Ltd, Llangunllo, Powys UK,*

[3] *Acumen Medical Ltd, Tiverton, Devon UK*

** Presenting and corresponding author: n.booth1@physics.ox.ac.uk*

Background: Cancer is one of the three major causes of death of people with Myalgic Encephalomyelitis or Chronic Fatigue Syndrome (ME/CFS). There is also anecdotal (and some trial) evidence that some cancer treatments (e.g. chemotherapy, Rituximab) mitigate the symptoms of ME/CFS. We report here on our studies of deficiencies in energy metabolism in neutrophils of people with ME/CFS, based on an audit of patients attending a private clinic specializing in this illness. Some of the results may be applicable to other illnesses.

Methods: Several hundred patients and 53 normal, healthy controls had the â€˜ATP Profileâ€™ test carried out on neutrophils (by JM-H at Acumen). This test yields 6 numerical factors that describe the availability of ATP and the efficiencies of oxidative phosphorylation and of the ADP-ATP translocator protein, TL (also called ANT, for adenine nucleotide translocator), in mitochondria. Other biomedical measurements, including the concentration of cell-free DNA in plasma and of some co-factors and antioxidants, were made.

Results: All patients tested have measureable mitochondrial dysfunction which correlates with the severity of the illness and with the level of cellular damage. Patients divide into two main groups differentiated by the nature of the dysfunction and how cellular metabolism attempts to compensate for it. Mitochondrial function is typically impaired in two ways: substrate or co-factor or antioxidant deficiency, and inhibition by chemicals, exogenous or endogenous. We find that apparent inhibition of the translocator protein TL is a major factor. A treatment regime, based on eating the evolutionary correct stone-age diet, ensuring optimum hours of good quality sleep, taking a standard package of nutritional supplements including antioxidants, getting the right balance between work and rest, and detoxification, improves mitochondrial function and clinical ability.

Conclusions: Biochemical measurements on mitochondria provide a valuable diagnostic tool and aid in the clinical management of ME/CFS. Apparent inhibition of the TL protein is a major factor and this may be relevant to some of the other illnesses that involve mitochondria and energy metabolism.

Notes:

Poster 4

Characterising the impact of novel regulators of mitochondrial DNA integrity on tumourigenesis

Gemma Bridge

Centre for Molecular Oncology, Barts Cancer Institute, Queen Mary University of London, London EC1M 6BQ

The extent to which cancer is caused by or is a consequence of mitochondrial genomic instability is unknown, but substantial data suggest an involvement of mutations in mitochondrial DNA (mtDNA) in the carcinogenesis process. A number of pathologies are also caused by mutations in nuclear genes that encode for mitochondrial proteins. Mutations in the nuclear-encoded components of the mitochondrial respiratory chain have been observed in cancer, for example in renal cell carcinoma. Until now there has been little direct evidence for a functional role of these various mutations. To assess the impact of global DNA damage on mitochondrial function we carried out a siRNA screen targeting 230 DNA repair and related genes in HeLa cells. The effect of each siRNA on mtDNA integrity was assessed after 5 days, by quantitative PCR (qPCR), to measure three mitochondrial genes: MT-CO1, MT-ATP6 and MT-ND2. This screen ascertained that silencing of MLH1, PMS1, MECP2 and REV3L caused a significant reduction in mtDNA integrity. We then independently validated these findings using two different siRNAs targeting each gene.

Our preliminary data suggest that knockdown of MLH1, PMS1 or MECP2 by siRNA renders HeLa cells more susceptible to treatment with H2O2 or the oxidative-damage inducing drugs Menadione and beta-lapachone, in comparison to control siRNA cells. This suggests a role for these genes in the oxidative stress pathway. This is further supported by data which suggests that reactive oxygen species (ROS) levels are higher in cells depleted of MLH1, PMS1, MECP2 or REV3L upon exposure to tert-butyl hydrogen peroxide (TBHP). We are currently investigating the impact that loss of these genes has on mitochondrial function, particularly on oxidative phosphorylation. We will also be attempting to ascertain the mechanisms by which these proteins control mtDNA integrity, by examining their subcellular locations and DNA binding properties.

Notes:

Poster 5

Wnt/?-catenin signaling as a novel mediator of Metabolism and Mitochondrial Dynamics in Melanoma

Kate Brown

UEA

The Royal Society

Wnts are secreted morphogens that play pivotal roles in embryonic development, stem cell biology and a number of disease states including cancer. Most Wnts signal through a pathway that results in the stabilisation of an intracellular signaling molecule called ÃŸ-catenin. In melanoma cells, Wnt/ÃŸ-catenin signaling has been implicated as a key regulator of cellular invasion and metastasis.

Using both transient and stable enhancement of Wnt/ÃŸ-catenin signaling, we demonstrate by confocal imaging that WNT3A facilitates perinuclear localisation of mitochondria and higher levels of mitochondrial networking in melanoma cells. Mitochondria of these cells are altered by Wnt/ÃŸ-catenin signaling to become perinuclear, highly networked and they show significant changes in the proteins of mitochondrial dynamics. Observed changes in mitochondrial fusion and fission proteins including MFN1, MFN2, OPA1 and DNM1L suggest that activation of Wnt/ÃŸ-catenin signaling can increase mitochondrial fusion and decrease mitochondrial fission in melanoma cells. Cellular metabolic analysis using the Seahorse Bioscience XFe96 Analyzer suggests that Wnt/ÃŸ-catenin mediated mitochondrial fusion may cause a global down-regulation of cellular energy metabolism in melanoma cells. This is supported by biochemical analysis of citrate synthase and lactate dehydrogenase activity.

We show that ?-catenin binds to the mitochondrial regulatory protein PARK2 in melanoma cells. Knockout of ?-catenin removes the mitochondrial fusion effect in these cells suggesting that it could be able to control mitochondrial dynamics.

In summary, we demonstrate that activation of Wnt/ÃŸ-catenin signaling in melanoma cells can lead to reduced cellular metabolism coupled with highly altered mitochondrial dynamics. This novel finding is controlled by ?-catenin has potentially wide implications for understanding how certain context-dependent effects of Wnt/ÃŸ-catenin signaling may be secondary to the regulation of mitochondrial dynamics and global cellular metabolism.

Notes:

Poster 6

Mitochondrial diaphorases-possible fuel engines for cancer cells thriving in a hypoxic environment?

Christos Chinopoulos

Department of Medical Biochemistry, Semmelweis University, Budapest, Hungary

Cancer cells thrive in hypoxic environments, harboring mitochondria with respiratory chain dysfunctions. As such, their mitochondria exhibit a decreased or complete loss of electron flux, associated with a diminished membrane potential. Under these conditions, ATP provision by the F0-F1 ATP synthase is thermodynamically impossible, and therefore ATP is provided by an overly activated glycolysis. Still, depolarized mitochondria pose the danger of being active ATP consumers, since the F0-F1 ATP synthase operates in reverse mode, and the inhibitory action of IF1 is not always applicable, due to cancer cell-specific expression. A potential bail-out mechanism of cancer cell mitochondria from cytosolic ATP dependence is generation of matrix high-energy phosphates by substrate-level phosphorylation, which may occur in the absence of oxidative phosphorylation. Substrate-level phosphorylation in mitochondria is almost exclusively attributed to succinyl-CoA ligase, an enzyme of the citric acid cycle which catalyzes the reversible conversion of succinyl-CoA and ADP (or GDP) to CoASH, succinate and ATP (or GTP). Provision of succinyl CoA by the alpha-ketoglutarate dehydrogenase complex (KGDHC) is critical for its maintained operation. However, for this mechanism to occur in view of a diminished -or absent- electron flux where NADH cannot be re-oxidized by complex I, NAD+ availability becomes a key limiting factor. Using pharmacologic tools and specific substrates and by examining tissues exhibiting no diaphorase activity, we show that mitochondrial diaphorases contribute up to 81% to the NAD+ pool during respiratory inhibition. Under these conditions, KGDHC's function, essential for the provision of succinyl-CoA to succinyl-CoA ligase, is supported by NAD+ derived from diaphorases. Through this process, diaphorases contribute to the maintenance of substrate-level phosphorylation during respiratory inhibition. Finally, we report that re-oxidation of the reducible substrates for the diaphorases was mediated by complex III of the respiratory chain.

Notes:

Poster 7

Mitochondrial DNA mutations are involved in the development of childhood myelodysplastic syndrome

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Introduction:

Normal cells contain different amounts of mitochondria which can simultaneously harbor wildtype and mutated mitochondrial DNA (mtDNA). Somatic mtDNA variants (either heteroplasmic or homoplasmic) have been found in 50-60% of adult MDS patients, with an increasing frequency with rising age and linearly rising incidences with advanced type MDS. As yet, no information is available on the frequency and role of mtDNA variants in pediatric MDS. We recently identified a pediatric MDS case in a family with (germline) mtDNA variants, and investigated whether and how often mtDNA variants occur in an extended cohort of childhood MDS.

Methods:

We analyzed the role of mtDNA variants in the index family, and in 19 childhood MDS patients, including sporadic primary, therapy-related and familial MDS, using the Mito-Chip2 (Affymetrix). The mitochondrial variants were validated by direct sequencing. Of the variants as defined by a frequency of less than 1% in the normal population in the different mitochondrial databases, a pathogenicity score was calculated. To investigate whether the variants were germline or somatic mtDNA mutation analysis was performed on DNA extracted from cultered fibroblasts or from saliva in MDS patients and their mothers. Oxidative phosphorylation assays were performed in the index family to evaluate the enzyme activity of the complexes.

Results:

In 13/19 (68%)pediatric MDS patients non-recurrent mtDNA variants were found versus 0.5% of the controls. Seven variants were scored as potential pathogenic. Mt-mutational status did not point towards a specific WHO subgroup of childhood MDS. Heteroplasmic variations were only found as somatic events, whereas germline variants were solely homoplasmic. Nine of the thirteen patients with mtDNA variants carried either additional cytogenetic or molecular aberrations.

Conclusion:

We describe the first family in which germline mtDNA variants triggered the development of pediatric MDS and show that also in non-familial pediatric MDS cases, somatic and germline variations frequently occur. This illustrates that MtDNA variants may cause an increased vulnerability for additional hits involved in the development of pediatric MDS.

Notes:

Poster 8

The RedMIT/GFP-LC3 mouse to monitor mitophagy

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Mitochondrial diseases are common and symptoms range from isolated presbyacusis, to loss of vision, to catastrophic neurodegeneration. Treatments are ineffective but mitophagy, mitochondrial recycling which is important in mitochondrial quality control, has been suggested in studies to have a role in neurodegenerative disorders such as Parkinsonâ€™s disease (PD). Growing evidences indicate that, in PD, accumulation of mitochondrial damage associated with excessive mitochondrial stress and impaired clearance of damaged mitochondria (mitophagy) lead to the loss of dopaminergic neurons in the substantia nigra, the midbrain region important for motor control. However this process has not been fully documented in vivo for technical reasons. An animal model, enabling visualisation and quantification of mitophagy, will overcome this major problem.

We made a novel mouse model (RedMIT) with dsRed targeted to mitochondria to visualise mitochondrial turnover. We confirmed that dsRed is targeted to mitochondria in tissues section and in mouse embryonic fibroblasts (MEFs) from this mouse. First results suggest that dsRed may slightly impair mitochondrial function, increasing to some extent the basal level of mitophagy. We have crossed the RedMIT mouse with GFP-LC3 homozygotes, expressing the hallmark of autophagy LC3 fused to the fluorescent protein GFP thus labelling the autophagosomal membrane. We used Imagestream (Amnis) and InCell system to investigate the co-localisation of mitochondria and the autophagic marker LC3 during active mitophagy in RedMIT/GFP-LC3 MEFs; we observed an increase in co-localisation with a chloroquine treatment or a depolarisation induced by CCCP. These results need to be reproduced in the animal to definitively validate our model.

Such a model will allow understanding of the underlying process of diseases implying a dysregulation of mitophagy.

Notes:

Poster 9

Effects of Doxorubicin on mitophagy and mitochondrial morphology

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Mitochondria have diverse functions within the cell, from supplying cellular energy, to signalling, cellular differentiation and cell death. Mitochondrial diseases that result from maternally transmitted mitochondrial DNA (mtDNA) mutations occur in 1/400 individuals. Cellular mechanisms for maintaining mitochondrial quality include mitophagy, and this could be a critical determinant of disease severity. In a previous study we showed that the anti-cancer drug doxorubicin remodels mtDNA [1.], a process that might underlie an unexpected side effect, delayed onset dilated cardiomyopathy (DCM). We reasoned that mitophagy might play an important role in the pathogenesis of DCM therefore we investigated the effects of doxorubicin on levels of mitophagy and mitochondrial morphology.

We used a previously developed high throughput imaging for quantifying mitophagy in cultured primary fibroblasts bearing the common pathogenic A3243G mtDNA mutation, associated with the mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episode syndrome (MELAS) and with diabetes mellitus and deafness.

We showed that doxorubicin increases mitophagy in fibroblasts which is appropriate in view of the mtDNA damage caused by the drug. We also saw mitochondrial elongation, consistent with the highly interlinked mitochondrial morphology that we described before [1.].

We conclude that mtDNA damage elicits increased mitophagy in response to doxorubicin which, concomitantly with an impaired mtDNA replication, could contribute to the mtDNA depletion we demonstrated. Mitochondrial interlinking is an adaptive response that raises cellular ATP levels in so called â€œstress induced mitochondrial hyperfusionâ€ [2.]. This is likely to counterbalance the increased level of mitophagy and might thus slow down the clearance of damaged mtDNA that is associated with delayed onset dilated cardiomyopathy associated with doxorubicin.

References:

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Notes:

Poster 10

TSPO and VDAC1 regulate a ROS-mediated pro-survival pathway by inhibiting Mitochondrial Quality Control

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The 18kDa Translocator Protein (TSPO) localises on the Outer Mitochondrial Membrane (OMM) and participates in the synthesis of cholesterol, it is also overexpressed in tumours and used to map their progression. We show that an altered expression ratio of TSPO to the Voltage Dependent Anion Channel 1 (VDAC1), to which it binds, triggers an accumulation of defective mitochondria that leads to apoptosis resistance and increased proliferative capacity. This is consequence of mitochondrial autophagy (or mitophagy) inhibition, which occurs downstream of the PINK1/Parkin pathway by preventing the required ubiquitination of OMM proteins.

Independent of cholesterol synthesis, the modulation of mitophagy by TSPO is instead dependent on VDAC1, through which it downregulates mitochondrial Ca2+ buffering capacity and ATP generation, whilst promoting an accumulation of Reactive oxygen species (ROS) which negatively affects Parkin-mediated ubiquitination of OMM proteins. This leads to an accumulation of dysfunctional mitochondria that are resistant to chemotherapeutic agents as well as an upregulation of ROSâ€“dependent Protein Kinases and Transcription Factors.

This set of data identifies TSPO as a novel element in the regulation of mitochondrial quality control by autophagy. It also demonstrates the importance of the expression ratio between TSPO and VDAC1 for cellular homeostasis and identifies a potentially critical element in oncogenesis and tumour progression.

Notes:

Poster 11

Mitochondrial IF1 preserves F1Fo-ATPsynthase enzymatic activity and cristae structure to limit ischaemic and apoptotic cell death

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Mitochondrial structure and dynamics play a central role in energy conversion and regulation of cell death. The ATPase Inhibitory Factor 1 (IF1) modulates the F1Fo-ATPsynthase enzymatic activity and oligomerization, protecting from ischaemic damage and contributing to formation of mitochondrial cristae.

Considering the emerging evidence for IF1 increased expression in human carcinomas, we explored its contribution to apoptosis. We found that IF1 over-expression has an anti-apoptotic role as it delays Cyt c release and ER-mitochondria Ca2+ cycle by preventing mitochondrial recruitment of the pro-fission protein Drp1 and the pro-apoptotic factor Bax. The protein therefore seems to take an active part in the preservation of the structural integrity of mitochondria during apoptosis, hindering fission. Hence, we also examined the effect of IF1 on the pro-fusion GTPase OPA1. It emerged that up-regulation of IF1 reduces Staurosporine-induced OPA1 processing, maintaining the physiological balance between long and short isoforms.

To further validate IF1 implication in ischaemia and apoptosis and elucidate its mechanisms of action, we designed two mutant forms of the protein: one has a non functional inhibitory domain (IF1 E30A) the other has a mutated hystidin rich region to prevent inactivation by oligomerization (IF1 H49P). Notably, IF1 E30A transfected cells showed higher rates of ischaemic cell death and apoptosis, whilst an opposite trend occurred in IF1H49P transfected cells.

This work confirms that IF1 has a critical role in the preservation of mitochondrial function and ultrastructure, improving the homeostasis of mitochondria and actively regulating their involvement in cell commitment to necrosis and apoptosis.

Notes:

Poster 12

Real-time assessment of the effect of acute and chronic hypoxia on cardiac metabolism in the control and diabetic rat: an in vivo study

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Currently over 340 million people worldwide suffer from type 2 diabetes(1), and as such it is crucial to understand the underlying aetiology, of which hypoxia may be a key aspect. Diabetics suffer microvascular complications, leading to reduced perfusion and oxygen delivery, and also have poorer prognosis after myocardial infarction(2), potentially due to compromised hypoxic signalling. Research into the metabolic response of diabetics to hypoxia may provide a new perspective for therapies.

This study investigated in vivo, real-time cardiac metabolism of both control and diabetic rats, subject to either an acute hypoxic insult or a chronic hypoxic environment. Hyperpolarized 13C pyruvate(3) was injected into anaesthetised animals in an MRI system. Sixty 13C spectra obtained over one minute enabled assessment of metabolism, using production of 13C bicarbonate as a measure of pyruvate dehydrogenase (PDH) flux. 13C lactate production was also analysed.

Acute hypoxia (30 minutes) caused a reduction in PDH flux, and a significant elevation of 13C lactate in control animals, neither of which was replicated in the diabetic group.

We showed a reduced diabetic cardiac PDH flux compared to controls in all experiments, however chronic hypoxia caused no further changes in either PDH flux or 13C lactate production. Hypoxia caused elevated plasma lactate in both groups expression of hypoxia-responsive proteins GLUT1 and PDK1 was unaffected.

Control animals were able to respond to the altered redox state induced by acute hypoxia, whereas diabetics were not. These data indicate a metabolic inflexibility in the diabetic heart.

In contrast, PDH flux was not affected by chronic hypoxia in either the control or diabetic heart, which we propose was due to physiological adaptations occurring and removing the stimuli for metabolic changes.

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Notes:

Poster 13

Mitochondrial pathways of cell death associated with Reactive Oxygen Species in Human Primary Glioma Cells

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Neuronal tissue is characterised by high concentrations of mitochondria, and brain is rich in membranes bearing polyunsaturated fatty acids. A diagnostic feature of high grade primary brain tumours (gliomas) are areas of necrosis, often associated with haemolysis. Necrosis and haemolysis of tissue give rise to reactive oxygen species (ROS) and lipid peroxidation, especially of polyunsaturated fatty acids. There has been increasing evidence of mitochondrial signalling of cell death in primary human brain tumours. This study aimed to analyse the effects of ROS on mitochondrial signalling in cells from primary human glioma.

The effects of reactive oxygen species (ROS) on mitochondrial associated cell death signalling were investigated in human glioma cells. Mitochondrial membrane potential sensitive probes were used to study sensitivity to ROS associated signals (H2O2, lipid peroxides, intracellular calcium) in cell preparations from 40 glioma patients (8 samples of peritumoral normal brain tissue were used as controls). This was supported by experiments on human cell lines in monolayer and spheroid culture and human primary glioma progenitor cell monolayers. Probes of intracellular peroxidation and cell death were also used.

Lipid peroxides were generated within minutes following the addition of 10-100uM polyunsaturated fatty acids to glioma cells, and this led to cell death within 6-18h, detected using TUNEL labelling and viability assays. Peroxidative signals also elicited rapid mitochondrial membrane depolarisation in a dose dependent manner and the kinetics of H2O2 and polyunsaturated fatty acid depolarisation were similar. Calcium ionophore, which mobilises membrane polyunsaturated fatty acids via phospholipase A2 activation, also elicited rapid mitochondrial membrane depolarisation.

This study indicated a pathway of glioma regression involving mitochondrial membrane depolarisation in response to ROS in human glioma cells. Mitochondrial membrane depolarisation was elicited by peroxidative stimuli and by exogenous polyunsaturated fatty acids. Selective activation of this signalling pathway may be relevant to therapeutic approaches to one of the most aggressive tumours known.

Notes:

Poster 14

Dysregulated mitophagy and mitochondrial transport in severe inherited opticatrophy due to OPA1 mutations

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OPA1 mutations are the commonest cause of dominantly inherited optic atrophy (DOA). They also cause phenotypes that are more severe, so-called DOA plus. While the Opa1 protein is essential for normal mitochondrial fusion, its role in the neurodegeneration underlying DOA remains unclear.

Profound down-regulation of Opa1 in control fibroblasts caused mitochondrial fragmentation, loss of mitochondrial DNA, impaired mitochondrial function and mitochondrial mislocalization, mediated by microtubules. We validated and used novel ImageStream technology to quantitate mitophagy in primary cells. Co-localization of mitochondria and autophagosomes was increased in fibroblasts in five patients from four families with severe OPA1 phenotypes, both at

baseline and after exposure to chloroquine, indicating increased mitophagy. ImageStream also showed that basal mitophagy was increased when control cultures were depleted of Opa1 by siRNA. Western blotting confirmed increased basal autophagy and autophagic flux in the presence of activators.

Increased mitochondrial fragmentation, mitophagy and failure of mitochondrial transport may together cause local depletion of mitochondria in critical regions of the retinal ganglion cells, such as axons or synapses. ragmentation may also impair stress induced mitochondrial hyperfusion. We have for the first time demonstrated increased mitophagy in primary human cultures, caused by a genetic disease.

Notes:

Poster 15

Theileria induces oxidative stress and HIF1a activation that are essential for host leukocyte transformation

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Complex links between infection and cancer suggest that we still can learn much about tumorigenesis by studying how infectious agents hijack the host cell machinery. We studied the effects of an intracellular parasite called Theileria which infects bovine leukocytes and turns them into invasive cancer-like cells. We investigated the host cells pathways that are deregulated in infected leukocytes and might link infection and lymphoproliferative disease. We show that intracellular Theileria parasites drive a Warburg-like phenotype in infected host leukocytes, characterized by increased expression of metabolic regulators, increased glucose uptake and elevated lactate production, which were lost when the parasite was eliminated. The cohabitation of the parasites within the host cells leads to disruption of the redox balance (as measured by reduced GSH:GSSG ratio) and elevated ROS (reactive oxygen species) levels, associated with chronic stabilisation of the hypoxia-inducible factor HIF1a. Inhibition of HIF1a (pharmacologically or genetically), or treatment with antioxidants, led to a marked reduction in expression of aerobic glycolytic genes and inhibited the transformed phenotype. These data show that stabilisation of HIF1a, following increased ROS production, modulates host glucose metabolism and is critical for parasite-induced transformation. Our study expands knowledge about the molecular strategy used by the parasite Theileria to induce the transformed phenotypes of infected cells via reprogramming of glucose-metabolism and redox signaling.

Notes:

Poster 16

The Resistant Cancer Cell Line (RCCL) collection

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The heterogeneity and individuality of cancer diseases is tremendously high. Recent genomic investigations revealed a tremendous genetic complexity in the cells from solid cancer diseases. Cancer cell (sub)populations may differ substantially between primary tumours and metastases as well as within primary tumours. This heterogeneity is a consequence of cancer clonal evolution processes. Among other models, comprehensive cancer cell line collections will be required to address this wide complexity.

Resistance acquisition to anti-cancer therapies represents a major obstacle to the development of effective anti-cancer therapies. Major cancer cell drug resistance mechanisms have been discovered in drug-adapted cancer cell lines including the ABC transporters ABCB1 (also known as P-glycoprotein or MDR1) and ABCC1 (also known as MRP1) and clinically relevant resistance mechanisms to so-called â€œtargeted therapeuticsâ€ (e.g. EGFR tyrosine kinase inhibitors, oncogenic BRAF inhibitors). The Resistant Cancer Cell Line (RCCL) collection consists of > 800 cell lines from 15 different cancer entities with acquired resistance to a broad range of cytotoxic and targeted anti-cancer drugs. It is a tool for the studying of drug-induced cancer cell resistance mechanisms including metabolic changes, the investigation of anti-cancer agents, and the examination of drug-induced clonal evolution processes.

Notes:

Poster 17

Embryonic stem cells become primed for cell death upon the onset of differentiation

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In the mammalian embryo pluripotent cells have the ability to contribute to all embryonic lineages and can be maintained in a naÃ¯ve state via the inhibition of exogenous growth factor signalling. The first identified step of differentiation of these pluripotent cells is the exit from the naÃ¯ve state and the acquisition of a primed pluripotent identity that is dependent on growth factor signalling. Both the naÃ¯ve and primed pluripotent states can be captured in vitro to give embryonic stem cells (ESCs) and epiblast stem cells (EpiSCs) respectively. ESCs and EpiSCs differ in their gene expression, signalling requirements and metabolism. To date, little is known regarding how cell survival is controlled in either the naÃ¯ve or the primed pluripotent states.

We have found that the transition from naÃ¯ve to primed pluripotency involves a critical change in the regulation of the apoptotic machinery and that this is likely to be associated with a change in stem cell metabolism. EpiSCs are dramatically more sensitive to death induced via the mitochondria than ESCs, what suggests that the intrinsic apoptotic machinery becomes primed for death upon differentiation. This is supported by the finding that EpiSCs but not ESCs are sensitive to the up-regulation of the pro-apoptotic protein Bim or the inhibition of the anti-apoptotic proteins Bcl2/Bcl-xL using ABT-737 . Furthermore, this shift in susceptibility to cell death signals appears to be metabolically controlled as inhibiting glycolysis with 2-deoxy-D-glucose, an analogue of glucose, makes ESCs become sensitive to ABT-737.

These studies provide evidence for a link between metabolism and apoptosis at the first stages of embryonic stem cell differentiation. Understanding the mechanisms by which stem cells change their apoptotic threshold during normal development not only contributes to our knowledge of stem cell biology and homeostasis, but could also represent a powerful model to give insight into the processes underlying cancer cell resistance to cell death stimuli.

Notes:

Poster 18

Targeting the mitochondria for the treatment of MLH1-deficient disease

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The DNA Mismatch repair (MMR) pathway is responsible for the repair of base-base mismatches and insertion/deletion loops that arise during DNA replication. MMR deficiency is currently estimated to be present in 15-17% of colorectal cancer cases and 30% of endometrial cancers. Furthermore, it has been shown that tumour cells can inactivate MMR genes as a mechanism of acquiring resistance to chemotherapies such as platinums and alkylating agents. Consequently, MMR deficient tumours are often resistant to standard chemotherapies, therefore there is a critical need to identify new therapeutic strategies to treat MMR deficient disease.

With the aim of identifying compounds that cause selective lethality upon MLH1 deficiency, we screened MLH1-deficient and proficient cells with a chemical library of 1120 compounds. Strikingly, upon analysis, a number of the hit compounds had previously been shown to target mitochondrial function. Our previous work has identified that silencing of a number of mitochondrial genes, including POLG and PINK1 are selectively lethal with MLH1 deficiency. Taken together this preliminary data strongly suggests that targeting the mitochondria may be a useful therapeutic strategy in MLH1 deficient disease.

To examine this further, we treated a panel of MLH1 deficient and proficient cell lines from a variety of tumour types with increasing concentrations of a range of mitochondrial targeted drugs in short term assays. We validated that the drugs Menadione, Parthenolide and B-Lapachone are all selectively lethal with MLH1 deficiency. These drugs are all known to induce reactive oxygen species (ROS) and oxidative stress amongst their mechanisms of action. Our data suggest, that specifically compounds that induce mitochondrial ROS and oxidative stress rather than targeting mitochondrial function in general, are selectively lethal with MLH1 deficiency.

To elucidate the mechanism behind this drug selectivity, we examined whether there are differences between mitochondrial function in MLH1 deficient compared to proficient cells. Initially, we examined the expression of oxidative phosphorylation complexes in the absence of MLH1 and observed a decreased expression of complex I (using the primary antibody NDUFB8, which detects an accessory subunit of Complex I) in the MLH1 deficient HCT116 cell line compared to its matched MLH1 proficient cell line HCT116+chr3. Based on these results, we carried out an ELISA assay for complex I activity in a panel of MLH1 deficient and proficient cell lines which also revealed a decrease in activity of complex I in the MLH1 deficient compared to proficient cell lines.

To establish whether there is a difference in oxidative phosphorylation and mitochondrial metabolism between the MLH1 proficient and deficient cell lines we used the Seahorse Extracellular Flux (XF) analyzer to determine any differences in the oxygen consumption rate (OCR) as a measure of oxidative phosphorylation in the HCT116 (MLH1 deficient) and HCT116+chr3 (MLH1 proficient) cell lines. We observed a significant decrease in the basal OCR and the spare respiratory capacity in the MLH1 deficient HCT116 cells compared to the MLH1 proficient HCT116+chr3.

Taken together, our results thus far suggest that oxidative phosphorylation and complex I activity is abrogated in MLH1 deficient cells, leading to decreased OCR and potentially resulting in the observed sensitivity to the oxidative stress inducing drugs, Menadione, Parthenolide and B-Lapachone.

Notes:

Poster 19

Very Low Doses of ?-Radiation Lead to Long Term Defects of Hematopoietic Stem Cells Functions

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After exposure to ?-irradiation, hematopoietic stem cells (HSCs) are more prone to survive than hematopoietic progenitors or mature hematopoietic cells. This radio-resistance is accounted for by a strong activation of p53-mediated DNA Damage Response, and by DNA repair through non-homologous end joining, an error-prone mechanism that leads to genomic instability in the long-term. These studies were focused on the effects of moderate and high doses of ?-irradiation on HSCs, but the effects of low doses of ?-irradiation (<0.1Gy) on HSCs are unknown.

Here, we report that ?-irradiation of HSCs at doses as low as 0.02Gy cause multiple long-term defects in HSCs functions such as a decrease of the LT-HSC pool in the reconstituted bone marrow of recipient mice after secondary transplantation and reduced tolerance to 5-FU treatment of the primary transplanted mice. In contrast with high doses, these effects were not accounted for by genomic instability.

This intrinsic radio-sensitivity of HSCs to low doses of ?-irradiation was not due to early apoptosis or DNA damage. Transcriptomic analysis of irradiated LT-HSCs showed that the early response to low doses of ?-irradiation specifically regulated a set of genes linked to the Keap1/Nrf2 pathway, to adhesion and to mitochondria function but independent of p53. Clonogenic assays performed with WT, Nrf2-/- and Keap1-/- HSCs showed the involvement of Keap1/Nrf2 pathway in the radio-sensitivity of HSCs to low doses of ?-irradiation and in vivo study showed that HSCs exposed to low doses of ?-irradiation displayed an initial abnormal homing that can be explain by the modification of tight junction genes expression. Finally, after low doses of ?-irradiation, genes encoding proteins involved in oxidative phosphorylation (OXPHOS) and citric acid cycle were up-regulated, indicating modifications of HSCs metabolism, modifications were associated with a higher mitochondrial activity of LT-HSCs. Interestingly, six months after transplantation of HSCs exposed to low doses of ?-irradiation, genes associated with mitochondria biogenesis and function together with genes involved in metabolic stress were deregulated in HSCs resulting in a persistent oxidative stress.

Altogether, these results showed, for the first time, the long term effects of doses of ?-irradiation as low as 0.02Gy on HSCs and suggested that low doses of ?-irradiation can impair HSCs function by altering mitochondria function, homing capacity and ROS detoxification. These alterations might prime the stem cell to differentiate rather than self-renew leading to an exhaustion of the HSCs compartment.

Notes:

Poster 20

Competitive elimination of defective cells during early mouse embryo development

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In a heterogeneous cell population, cells are able to compare their metabolic properties, signalling abilities or growth rates. This cell-cell interaction, known as cell competition, results in the growth of the stronger population at the expense of the weaker one.

We have found that cell competition monitors cellular fitness in the early post-implantation mouse embryo. We observed that mouse embryonic stem cells with impaired BMP signalling, deficient autophagy or with a tetraploid karyotype are out-competed by wild-type cells at the onset of differentiation. Concurrent with the elimination of defective cells, wild type cells undergo compensatory proliferation, in this way ensuring homeostasis of the overall cell population. Key to the elimination of unfit epiblast cells is the establishment of differential expression levels of the proto-oncogene c-Myc, leading to the apoptotic elimination of those cells with lower c-Myc expression both in vitro and in the early post-implantation mouse embryo. We will discuss the importance of signalling and metabolic events that determine relative cellular fitness levels during cell competition in the early mammalian embryo.

By allowing the recognition and elimination of weaker cells, cell competition provides a mechanism that could control cellular fitness in a wide variety of settings, including stem cell and tissue homeostasis, organ size or the expansion of precancerous cell fields.

Notes:

Poster 21

Mitochondrial targeting of cyclosporin analogues

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Neurodegeneration is a major factor in the underlying disease progression of MS contributing to increasing disability.

The mitochondrial permeability transition (PT) pore, is now recognized as a key player in the degeneration of axons. The mitochondrially localized peptidylprolyl cis-trans isomerase, cyclophilin D (CyP-D), has been shown to be important in the function of the PT pore. Knock out mouse studies in multiple sclerosis models have shown that genetic ablation of CyP-D provides neuroprotection. The PT pore is found in human brain, is sensitive to block by cyclosporin A (CsA) and is also a target for other neurodegenerative conditions.

Clinical trials of CsA have been reported in traumatic brain injury, where a low dose was utilized to minimize the known toxic effects. Despite low concentrations of CsA in the brain, an improved clinical score was noted. Likewise trials of CsA in multiple sclerosis produced some modest benefit in progressive MS, but were hampered by dose limiting side-effects. However as we shall show CsA is however a poor neuroprotectant exhibiting a bell shaped dose response curve.

We describe new selective cyclosporine based inhibitors of mitochondrial cyclophilin that avoid the immunosupression of CsA, and much of the known toxicity by avoiding interaction with the cytoplasmic cyclophilins. A detailed modelling design is combined with organic synthesis to yield inhibitors that are characterized with a range of biochemical assays. We are currently progressing these compounds through in vivo testing in models of MS disease.

Notes:

Poster 22

Wnt S

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Altered metabolism and mitochondrial behaviour has been well documented in cancer cells and these changes are essential to fuel oncogenic behaviours such as enhanced proliferation and migration, which in turn facilitate tumour progression. Therefore, altered cell metabolism in tumours provides an opportunity for novel therapeutic intervention. Aberrant control of Wnt signalling has been identified in a large number of cancer types. Recently, links have emerged between Wnt signalling and altered cell metabolism in cancer cells, but relatively little is known about how Wnt signalling coordinates the metabolic activity of tumours. We have found that both beta-catenin-dependent and -independent Wnt signalling pathways can coordinate changes in cancer cell metabolism and mitochondrial activity in a highly context dependent manner. For example in a subset of melanomas (an aggressive and often highly metastatic form of skin cancer), Wnt-beta-catenin signalling can reduce cell migration with concomitant remodelling of the mitochondria forming highly fused networks. This change to the mitochondria provides a characteristic metabolic phenotype, which we hypothesise is linked with a reduced propensity of the melanoma cells to metastasise. However opposite effects are seen with beta-catenin-independent Wnt signalling in these cells. Overall, we propose that Wnt signalling pathways play a pivotal role in reprogramming tumour cell metabolism.

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Poster 23

An anti-tumorigenic effect ascribed to severe respiratory complex I mutations

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In order to sustain tumor growth cancer cells have to undergo a metabolic switch from oxidative metabolism toward glycolysis, the so-called Warburg effect.

The aim of this work is to prove that functional respiratory complex I is necessary to complete the glycolytic shift, making it essential to support tumor progression during the hypoxia response.

As a consequence, severe complex I (CI) mutations may confer anti-tumorigenic properties.

Cells and tumors lacking complex I activity by high loads of mitochondrial DNA mutations displayed reduced tumor growth and invasiveness compared to those harboring lower mutation loads, defining a threshold level for an anti-tumorigenic effect of such genetic lesions.

The recovery of a functional enzyme via allotopic expression reverts the phenotype observed and is required to induce a Warburg profile, promoting cancer progression.

Such trigger is mediated by the stabilization of the hypoxia inducible factor 1a (HIF1a), due to the recovery of the balance between a-ketoglutarate and succinate permitted by a recuperation of NADH consumption that follows complex I rescue.

Henceforth, we propose to prevent CI activity by knocking-out the expression of NDUFS3, a crucial CI assembly subunit, in cancer cell lines using the zinc-finger nucleases technology. With this approach we expect to record a decreased tumorigenic and metastatic potential of knock-out clones compared to CI-competent cells, revealing the metabolic changes previously characterized.

Here, the creation of the NDUFS3 knock-out cell line will be presented.

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Notes:

Poster 24

Tumour suppressing and tumour progressing activities of MnSOD

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The mitochondrial enzyme Manganese Superoxide Dismutase (MnSOD) is a mitochondrial matrix protein that catalyses the dismutation of superoxide radicals to hydrogen peroxide. MnSOD has been shown to have two faces with regard to its role in tumour development. On the one side it is well documented that overexpression of MnSOD slows down cancer cell growth, whereas on the other side MnSOD also has a metastasis promoting activity. Based on the growth retarding effect of MnSOD it has been hypothesized that MnSOD might be a tumour-suppressor gene and that restoring MnSOD activity should reverse the malignant phenotype of tumour cells. However, quantitative analyses revealed that colorectal carcinomas and liver metastases expressed up to 4 times more MnSOD than normal mucosa and that adenomas expressed intermediate MnSOD levels that increased significantly with the grade of dysplasia. Moreover, it has been shown that a high MnSOD content of colorectal carcinomas was associated with poor 5-year overall survival of the patients. Similar findings were obtained with other types of cancer highlighting the association of MnSOD with metastasising and late-stage tumour disease. Our goal is to elucidate mechanistic explanations for these seemingly contradictory behaviours of MnSOD, in order to find specific intervention points in the respective pathways.

With respect to the growth-retarding effect, we could demonstrate that overexpression of MnSOD slowed down growth of HCT116 human colorectal cancer cells by induction of cellular senescence. Experiments in isogenic p53 and p21 knock-out cells revealed that this effect was p21-independent, but required p53. Analysis of the mitochondrial membrane potential demonstrated reduced polarisation in MnSOD overexpressing cells. In addition, forced depolarisation of the mitochondrial membrane by mitochondrial inhibitors like rotenone or antimycin-A also led to p53-dependent senescence. Other research teams found that in primary keratinocytes the NF-kappaB transcription factor c-Rel causes MnSOD upregulation and subsequent onset of senescence. Furthermore, it was demonstrated that down regulation of phosphoglucose isomerase/autocrine motility factor (PGI/AMF) leads to oxidative stress-induced cellular senescence and that this process is driven by elevated MnSOD levels. Hence, the role of MnSOD in senescence has been established in various different models. However, the exact mechanistic steps from MnSOD activity to onset of senescence remain to be elucidated in more detail.

Additionally, we could show that MnSOD contributes to resistance to tumour-necrosis-factor-related-apoptosis-inducing ligand (TRAIL)-induced apoptosis. This protective effect is p53-independent. MnSOD prevented release of proapoptotic factors such as cytochrome c and SMAC/Diablo from mitochondria into the cytosol in TRAIL-treated cancer cells, thereby inhibiting activation of effector caspases and cell death. Interestingly, in TRAIL knock-out mice experimental tumours grow more aggressively and tend to spread to other tissues more readily pointing to a substantial role of TRAIL as a tumour surveillance factor that is able to eliminate developing and disseminating tumours. Thus, it appears possible that MnSOD-induced senescence and MnSOD-induced apoptosis resistance are working in opposite directions leading to growth arrest on the one side and facilitating tumour metastasis on the other. Alternatively, slow growth/senescence and apoptosis resistance might act in concert providing a protective shield under which cancer cells can better disseminate and survive in distal organs. Indeed, when we injected normal HCT116 cells and HCT116 cells overexpressing MnSOD into mice, we unexpectedly found that the MnSOD-overexpressing cells grew out more aggressively than their parental counterparts hinting that MnSOD expression can give rise to better seeding and growth in ectopic sites. Further detailed analyses are required to clearly attribute the different MnSOD functions to tumour behaviour in vivo, which can then be potentially targeted in new cancer therapies.

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