A fresh look at cancer metabolism in a historical setting

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The Institute for Research in Biomedicine (IRB) Barcelona meeting on Cancer Metabolism took place in November 2010. It was organized with the support of the Banco Bilbao Vizcaya Argentaria Foundation and offered an outstanding line-up of acclaimed speakers within an intimate setting that allowed plenty of opportunity for informal interactions.

The historic heart of Barcelona formed the backdrop for this gathering to discuss recent advances in the field of cancer metabolism. The conference was organized by Joan Guinovart (IRB, Spain) and Dario Alessi (Medical Research Council, Dundee, UK) and boasted an impressive range of interesting talks, as well as outstanding poster presentations. The stunning conference venue was matched by the excellent food and an engaging introduction to Catalan culture by local host Joan Guinovart.

Barcelona's wonderful mix of historical buildings and modern architecture seems to illustrate the revival of interest in cancer metabolism. This field was founded in the first half of the last century by Otto Warburg's observation that cancer cells use glycolysis, rather than oxidative phosphorylation, for energy production. Over the past 40 years, much of the research into the molecular causes of cancer has focused on oncogenes and tumour suppressors. However, the analysis of cancer metabolism has recently been gathering momentum, through the application of state-of-the-art molecular, genetic and biochemical techniques.

More than proliferation

The meeting started with a stimulating presentation from Salvador Moncada (Wolfson Institute, London, UK), who addressed the relationship between metabolism and cell-cycle regulation in normal cells. In collaboration with Juan Bolaños and Angeles Almeida (University of Salamanca, Spain), Moncada found that astrocytes respond to

the inhibition of mitochondrial energy production by inducing PFKFB3, an isoform of the bi-functional PFK2 enzyme. PFKFB3 synthesises 2,6-bisphosphate, which is a potent allosteric activator of phosphofructokinase 1 (PFK1). The team uncovered a new mechanism for post-transcriptional regulation of PFKFB3 by the APC/c-cdh1 ubiquitin ligase. PFKFB3, but not other PFK2 isoforms, contains a KEN-box, which mediates APC/c-cdh1-dependent degradation of cell-cycle proteins (Herrero-Mendez *et al*, 2009). Moncada also found that glutaminase 1—a crucial enzyme in cell

proliferation—is under the control of APC/c-cdh1, suggesting that this might be the mechanism that coordinates cell-cycle progression with the metabolic changes required to support it (Colombo *et al*, 2010).

The coordination of metabolic activity with cell-cycle progression is crucial to support the proliferation of normal cells. Deregulation of cell-cycle control in cancer could cause some of the metabolic adaptations observed in cancer cells. The importance of regulating glycolytic flux in cancer cells was illustrated by Karen Vousden (Beatson Institute, Glasgow, UK). Her group has identified TIGAR as a transcriptional target of the p53 tumour suppressor. TIGAR functions as a fructose-2,6-bisphosphatase (FBPase), reducing the levels of fructose-2,6-bisphosphate, which results in decreased glycolytic activity and increased flux through the pentose-phosphate pathway. This activity of TIGAR is important to maintain cellular redox balance and its depletion results in increased levels of reactive oxygen species. Vousden also discussed functions of TIGAR that are independent of its FBPase activity, as well as its expression in colon-cancer samples. The exact role of TIGAR in tumorigenesis will become clearer from studies using knockout mice.

The high glycolytic rate of cancer cells in low-oxygen conditions produces excess lactate, which causes acidification and inhibition of pH-sensitive glycolytic enzymes. Jacques Pouyssegur (U. Nice, France) demonstrated the importance of pH regulation for tumour growth by showing that deletion of NHE1, a sodium-dependent proton transporter, prevents tumour formation

Fig 1 | The balance between metabolic demand and nutrient supply in cancer cells. Metabolic regulators such as AMPK balance metabolic activity with nutrient availability. (A) Rapid proliferation of cancer cells requires the synthesis of macromolecules such as nucleotides, proteins and lipids. These energy-consuming processes are downregulated under conditions of reduced nutrient supply while energy production through oxidation of lipids is activated. (B) Severe nutrient depletion can also cause the degradation of cellular components through the process of autophagy. (C) Disruption of metabolic regulation in cancer cells, for example by inactivation of AMPK, causes an imbalance between demand and supply, and results in cell death.

in respiration-deficient Ras-transformed fibroblasts. In addition, deletion of carbonic anhydrases IX and XII (CA9 and CA12)—two extracellular enzymes that convert carbon dioxide and water into carbonate, resulting in the acidification of the extracellular milieu—causes severe loss of viability in cancer cells (Chiche *et al*, 2009), demonstrating the importance of pH regulation in cancer cell survival.

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Another metabolic challenge for glycolytic cancer cells is the removal of excess lactate. Lactate export is facilitated by the monocarboxylate transporters (MCT1–4). Pouyssegur showed that combined depletion of both MCT1 and MCT4 blocks the proliferation of colon-cancer cells and delays the onset of xenograft tumours, but is not sufficient to induce cell death and tumour regression. Pouyssegur suggested that cancer cells respond to MCT inhibition by shutting down energy-consuming

processes, probably by engaging metabolic regulators such as AMPK. Maintaining the balance between energy demand and nutrient supply is crucial for the survival of cancer cells. Limiting the supply of nutrients while forcing energy-consuming processes to remain active could be a strategy for cancer therapy. This creates an imbalance between supply and demand resulting in the collapse of tumour growth (Fig 1).

Celeste Simon (U. Pennsylvania, USA) discussed the wider role of the hypoxic microenvironment. Activation of Hif1 under hypoxia supports adaptation to this metabolic challenge. While hypoxia is generally associated with the tumour microenvironment, it is also an important feature of the niche of certain stem cells. Simon's group showed that Hif1 regulates Wnt signalling by driving the expression of the Lef1 and TCF1 transcription factors. Crucially, activation of Hif1 was required for hippocampal neuronal development in mice, suggesting that it is important for the maintenance of neuronal stem-cells (Mazumdar *et al*, 2010).

mTORC1 and lipid synthesis

Two talks discussed the importance of lipogenesis in the context of cancer metabolism. Enhanced lipogenesis is frequently observed in human tumours, but the understanding

of its role in tumour development is limited. Almut Schulze (Cancer Research UK London Research Institute) showed that the SREBP transcription factor is activated by Akt in a mTORC1-dependent manner. Schulze's group found that activation of SREBP supports Akt-dependent growth in tissue culture cells and is required for cell and organ growth in flies (Porstmann *et al*, 2008). Interestingly, her group also found evidence for crosstalk between lipid and protein synthesis downstream from mTORC1, suggesting that both processes have to be regulated in a concerted manner to support cell growth and proliferation.

Brendan Manning (Harvard U., USA) explored the regulation of lipogenesis by the mTORC1 pathway. His group has previously shown that SREBP is an important transcriptional regulator downstream from mTORC1 signalling (Duvell *et al*, 2010). Here he presented his recent findings on the regulation of lipogenesis by mTORC1 *in vivo*. Spontaneous kidney tumours arising in TSC2-heterozygous mice show increased expression of SREBP target genes. Interestingly, treatment with a single dose of rapamycin was sufficient to lower the expression levels of these genes, confirming that SREBP is regulated by mTORC1 *in vivo*. However, the exact role of SREBP and its target genes in mTORC1-dependent tumorigenesis is not yet clear. Future work is required to unravel the interaction between lipid synthesis and cancer development.

Keeping control: AMPK and cancer

The LKB1/AMPK pathway took centre stage, with several speakers discussing different aspects of this signalling pathway. Dario Alessi presented insights obtained from an analysis of the structure of the complex between LKB1, STRAD α and MO25 α . His study showed that STRAD α binds to LKB1 as a pseudosubstrate and that binding of MO25 and STRAD α stabilizes the open conformation of LKB1 by binding to its activation loop. This work suggests that allosteric activation by the binding of pseudokinases might represent a mechanism that is widely used for the regulation of kinase activity, as approximately 10% of all mammalian kinases lack catalytic activity. The study demonstrated how mutations in LKB1 found in cancer impair its catalytic activity and its interactions with substrates and regulators.

Grahame Hardie (U. Dundee, UK) discussed the role of AMPK in cancer. AMPK is a master regulator of cell metabolism

and modulates the activity of many catabolic and anabolic processes. Rapid proliferation and limited nutrient supply are likely to cause chronic activation of AMPK in cancer cells, thereby inducing a strong selective pressure to inactivate this pathway. Several types of cancer show inactivation of the AMPK pathway through loss of LKB1 or downregulation of AMPK expression. Different therapeutic strategies therefore aim to reactivate AMPK (either directly or indirectly) in cancer. Hardie also discussed that viruses such as hepatitis C (HCV) inactivate AMPK to enhance the replication of their genome by the host cell (Mankouri *et al*, 2010). The inactivation of AMPK following HCV infection could thus contribute to cell transformation during the development of hepatocellular carcinomas.

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Several speakers discussed the search for new targets of the AMPK-signalling pathway. Anne Brunet (Stanford U., USA) discussed her proteomics approach to identify new AMPK phosphorylation targets. Her group used the affinity-tag method developed by Kevin Shokat (UC San Francisco, USA) that allows the capture of phosphates transferred by a specific kinase. First, they modified the ATP-binding pocket of the α 2-kinase subunit of AMPK to bind to a specific ATP analogue. Proteins phosphorylated by this kinase can be identified by using antibodies detecting the modified phosphate. Brunet's group used this method to capture AMPK substrates from cells treated with 2-deoxyglucose to mimic energy starvation. AMPK substrates are likely to include important mediators of the effect of dietary restriction on metabolism, tumour suppression and lifespan regulation.

Kun-Liang Guan (UC San Diego, USA) and Reuben Shaw (Salk Institute, USA) both reported that AMPK phosphorylates ULK1, an important regulator of cellular autophagy. Guan showed that phosphorylation of ULK1 by AMPK increases ULK1 kinase activity and is required for autophagy induction under conditions of energy restriction. He presented data showing that mTORC1 phosphorylates ULK1 and this phosphorylation disrupts the interaction

between ULK1 and AMPK, therefore preventing ULK1 activation (Kim *et al*, 2011). Shaw showed that AMPK-dependent phosphorylation of ULK1 and ULK2 prevents the accumulation of damaged mitochondria in the liver. He concluded that AMPK induces ULK-dependent degradation of damaged mitochondria (mitophagy), and at the same time induces mitochondrial biogenesis through activation of PGC1 α and PPAR δ —a process that he aptly compared to the 'cash for clunkers' programme for old cars in the USA.

Shaw went on to discuss the use of anti-diabetic drugs as potential cancer therapeutics and the results of tests of this approach in human non-small-lung cancer cell lines and *in vivo* in K-Ras-dependent genetically engineered models of lung cancer. The results suggest that failure to activate AMPK might make cancer cells more susceptible to the effects of nutrient or energy limitation. They also reveal the somewhat contradictory role of AMPK in cancer development and maintenance. Loss of AMPK signalling promotes tumorigenesis, whereas established tumours might require AMPK to maintain their energy balance.

The complexity of cancer metabolism

There is increasing evidence that genetic alteration of metabolic genes could be causative for cancer development. Valeria Fantin (Agiros Pharmaceuticals, USA) gave an overview of the identification of mutations in isocitrate dehydrogenase (IDH1 and IDH2) in low-grade gliomas and secondary glioblastoma, as well as acute myeloid leukaemia. These mutations are heterozygous point-mutations in arginine residues within the active site, suggesting that they have a gain-of-function effect. Structural studies revealed that mutant IDH has a similar structure to the wild-type enzyme in its active conformation. Interestingly, mutant IDH acquires a neomorphic activity and catalyses the conversion of α -ketoglutarate into 2-hydroxyglutarate (2HG; Dang *et al*, 2009; Ward *et al*, 2010). Although production of 2HG by tumours harbouring mutations in IDH offers new diagnostic opportunities, its exact role in cancer is still open to speculation.

David Sabatini (Whitehead Institute, USA) discussed his recent results on the regulation of mTORC1 by amino acids. A trimeric complex consisting of MP1, p14 and p18 (termed Ragulator) mediates the localization of mTORC1 to the

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lysosomal surface (Sancak *et al*, 2010). He also reported on a systematic approach to addressing the complexity of cancer metabolism. His group used short-hairpin RNA screening to identify genes that support the growth of a breast-cancer cell-line *in vivo*. This work demonstrates that the complex nature of metabolic networks has to be considered in the analysis of the metabolic requirements of cancer cells.

Adding another layer of complexity to metabolic regulation, Kun-Liang Guan touched on the importance of acetylation of metabolic enzymes, which is evolutionarily conserved and can cause activation or inhibition of enzymatic activity. Guan equated the impact of protein acetylation to that of other post-transcriptional modifications, such as phosphorylation in cellular signalling and ubiquitination in cell-cycle regulation.

The scope and diversity of the topics discussed at this excellent meeting demonstrate the progress that has been made in appreciating the complexity of cancer metabolism since Warburg's discoveries. However, they also show that much of the metabolic tangle is still waiting to be unravelled.

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