Metabolism and cancer mix in Madrid

Reuben J. Shaw

The CNIO Cancer Conference 'The Energy of Cancer' held last November was a bridge between metabolic and cancer research, emphasizing the interdisciplinary nature of the field. It confirmed that the outlines of the main pathways involved in coordinating nutrient uptake and metabolism with cell growth have begun to emerge.

Although altered glucose metabolism was first noted as a characteristic of tumour cells by Otto Warburg in the 1920s, the molecular underpinning for this observation has not begun to be appreciated fully until the past decade. However, extensive interactions between genuine tumour suppressor genes and oncogenes and metabolic control have been found in the past five years. Teleologically, it makes sense that cells need to coordinate metabolic and proliferative control, but the biochemical switches that govern this coordination have only begun to emerge. The CNIO Cancer Conference 'The Energy of Cancer', organized by Toren Finkel (NIH), David Sabatini (Whitehead Institute/MIT, Cambridge, USA), Manuel Serrano (CNIO, Madrid) and David Sinclair (Harvard Medical School), was held in Madrid, Spain, between 2 and 4 November 2009. The conference was a forum for the discussion of the latest advances in this area, and of how this knowledge could lead to future cancer therapeutics. More than 200 attendees—a combination of basic researchers in the metabolic disease field, as well as in cancer and cell biology—also had the honour of the attendance of James D. Watson (Cold Spring Harbour Laboratory) who gave an address denoting his renewed interest in the area of cancer and metabolism. His talk was peppered with anecdotes and perspective on how this topic evolved from being the cornerstone of cancer research in the pre-DNA days of studying the viral nature of tumorigenesis, all the way up to its resurgence today.

The mTOR and AMPK pathways

The increased demand of ATP and nutrient resources for biosynthesis and proliferation in growing cells requires that signals sensing and integrating nutrient cues be coupled to the growth control machinery. A central regulator governing the growth of all eukaryotic cells is target of rapamycin (TOR), which in mammals is found in two complexes: the mammalian TOR complex 1 (mTORC1) and mTORC2. Complex 1 contains the TOR serine/threonine kinase along with its scaffolding partner raptor and associated subunits; it is nutrient-sensitive, acutely inhibited by rapamycin and phosphorylates S6K1 and 4EBP1 to control protein translation. The mTORC2 complex is composed of TOR and rictor, and phosphorylates the hydrophobic motif of a number of AGC family kinases, including Akt.

Studies from many groups have delineated a signalling pathway upstream from mTORC1, which is deregulated through various mechanisms in most human cancers. The activity of mTORC1 is dependent on the small Ras-like GTPase Rheb, the GTP-loaded state of which is regulated by a

GTPase-accelerating protein (GAP) complex composed of the TSC1 and TSC2 tumour suppressors. Akt, Erk and Rsk can phosphorylate and inactivate TSC2 after growth factor stimulation or in cancer cells with hyperactivated Ras or PI(3)K pathways, leading to the activation of mTORC1. Recent advances have also begun to illuminate how the mTORC1 pathway senses amino acids and how this signal is integrated with growth factor inputs to mTORC1. Sabatini described a family of small GTPases, the Rags,

which interact with mTORC1 in an amino-acid dependent manner. Amino acids were shown to induce the translocation of raptor to an endosomal subcellular localization where the Rheb GTPase is located. Chimaeras of raptor and the carboxyl terminus of Rheb localized permanently to these vesicles, even in the absence of amino acids, and mTORC1 signalling was no longer responsive to changes in the Rag GTPases or amino acids. Collectively, these data suggest that the Rag GTPases translocate the raptor–TOR complex to a vesicle compartment containing Rheb when amino acids are present, thus explaining how the amino acid input coordinates with the growth-factor-dependent signals that act through Rheb. Lisa Henske (Brigham and Women's Hospital, Boston) discussed two disorders that are caused by a loss of TSC2 and characterized by the hyperactivation of the mTORC1 pathway: tuberous sclerosis (TSC) and lymphangioleiomyomatosis. One of the crucial processes regulated by mTORC1 is the suppression of autophagy, thus cells that lack TSC2 are particularly deficient in this process. Henske described

ongoing efforts to use autophagy modulators to inhibit the growth of TSC-deficient cells. Brendan Manning (Harvard U.) described a systems biology approach to detail the transcriptional and metabolic outputs that occur downstream of mTORC1 by using TSC-deficient cells treated with rapamycin. He highlighted that different aspects of glucose metabolism are controlled by distinct transcriptional outputs downstream of mTORC1. Further exploring the organismal control of metabolism through the mTORC1 and PI(3)K pathway, Mario Pende (U. Paris Descartes) described some of the metabolic phenotypes of mice lacking Pten, S6K1, or Akt2. Akt2 was uniquely found to have a crucial role in a number of metabolic and tumour-dependent phenotypes in Pten-deficient animals.

The energy-sensing AMP-activated protein kinase (AMPK) pathway balances the anabolic pro-growth signals of the mTORC1 pathway. AMPK is activated by the LKB1 serine/threonine kinase when intracellular ATP levels are low, such as under nutrient-poor conditions. Dario Alessi (U. Dundee) described the crystal structure of LKB1 bound to its two regulatory subunits and discussed a novel effector of the LKB1 pathway in a phosphatase complex that controls myosin phosphorylation and cell adhesion. Jay Brenman (U. North Carolina, Chapel Hill) reported the characterization of flies deficient in AMPK. Neuronal loss of AMPK leads to neurodegenerative defects and systemic loss of AMPK has effects that are exacerbated by nutrient deprivation in both embryos and adults. Consistent with these *Drosophila* AMPK mutant phenotypes and AMPK activation in nutrient-poor conditions, Anne Brunet (Stanford U.) reported that AMPK has a crucial role in the response to stress and in dietary restriction-induced lifespan extension in *Caenorhabditis elegans*. This phenotype is at least partly due to the phosphorylation of Foxo family transcription factors by AMPK. Reuben Shaw (Salk Institute) discussed the aforementioned TOR binding partner raptor as another conserved substrate of AMPK that has crucial roles in nutrient response and cell growth. Raptor phosphorylation by AMPK inhibits mTORC1 signalling, enforcing a metabolic checkpoint in the cell. These and related findings suggest that existing drugs that activate AMPK, including the widely used diabetes therapeutic metformin, could be used as anti-cancer agents. Michael Pollak (McGill U., Montreal) also

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discussed epidemiological studies that correlate metformin treatment with reduced cancer risk. Systemic metformin treatment can suppress tumour cell growth both cell-autonomously—through AMPK-dependent suppression of mTORC1—and also through non-cell-autonomous effects, by lowering circulating insulin and IGF1 levels in response to lower circulating blood glucose brought about by AMPK effects in the liver. Consistent with this hypothesis, murine tumour models fed a high-fat diet had lower insulin and decreased growth rates in response to metformin treatment.

Transcriptional metabolic regulators

In response to alterations in mitogenic signalling pathways, cells alter their transcriptional programmes to re-engineer their metabolic consumption. HIF1 α is a transcription factor that is increased by protein stabilization under hypoxia, the translation of which is critically dependent on mTORC1 and seems to be upregulated in various cancers. HIF1 α induces the expression of several target genes, prominent among which are angiogenic factors and isoforms of every step of the glycolysis pathway and negative regulators of oxidative phosphorylation. Thus, through the upregulation of HIF1 α , tumour cells enhance glycolysis at the expense of mitochondrial oxidative phosphorylation, providing—at least in part—a molecular basis for the aforementioned Warburg effect. Gregg Semenza (Johns Hopkins U., Baltimore) described the results of screens for drugs that inhibit HIF1-mediated transcription. Interestingly, two classes of existing pharmaceutical were isolated as inhibitors of HIF1: cardiac glycosides—such as digoxin—which are used to chronically treat patients with heart disease, and anthracyclines—such as the widely used chemotherapeutic doxorubicin—which act through a different mechanism to inhibit HIF1 function. Peter Carmeliet (Vesalius Research Center, Leuven, Belgium) discussed the phenotypes of mice deficient in PHD enzymes, emphasizing the role of HIF in cell survival and angiogenesis under oxygen-limiting conditions. Jacques Pouyssegur (Centre Antoine Lacassagne, Nice) focused on HIF1 targets involved in pH control; by using cells deficient in

carbonic anhydrases and monocarboxylate transporters, he showed that they contribute significantly to the tumorigenic phenotype, placing them as new potential targets for anti-cancer therapeutics.

Another crucial oncogene that controls cellular metabolism is the basic helix–loop–helix transcription factor c-Myc. As discussed by Chi Dang (Johns Hopkins U., Baltimore), Myc reprogrammes glucose metabolism through genes that are also HIF1 α targets—such as LDHA—suggesting that they would be particularly good drug targets. In addition, Myc redirects glutamine metabolism through microRNA-mediated modulation of glutamine transporters and glutaminase. A related Myc family member, ChREBP is highly expressed in metabolic tissues such as liver and adipocytes, as discussed by Barbara Kahn (Harvard Medical School). ChREBP senses glucose directly and transactivates genes involved in fatty acid synthesis. Bruce Spiegelman (Harvard Medical School) discussed PRDM16, a novel transcriptional co-activator involved in the conversion of brown fat and required for specification of the brown fat lineage from a myoblastic precursor. Transplantation of fibroblasts with enforced expression of PRDM16 and its transcriptional partner C/EBP β convert them to brown-fat-like cells, endowing them with the ability to dramatically increase glucose uptake, as visualized by FDG-PET. Interestingly, PRDM16 was originally found as a translocation fusion in cases of chronic myelomonocytic leukaemia and acute myeloid leukaemia, suggesting that reprogramming its transcription might also alter survival or growth pathways in some lineages.

Finally, Toren Finkel (NIH) described that mice deficient in the polycomb stem cell renewal factor BMI1 are strikingly defective in mitochondrial function and have increased reactive oxygen species production, which results in the activation of the DNA damage pathway and premature ageing phenotypes. These results suggest that polycomb proteins coordinately regulate transcriptional programmes that determine stem cell fate with those of cellular metabolism.

Sirtuins are class III histone deacetylases that also deacetylate other proteins, including a number of transcriptional regulators. As they depend on NAD⁺ for enzymatic activity, sirtuins act as independent sensors of cellular energy status, which in turn reprogramme cell metabolism and cell fate. Johan Auwerx (École Polytechnique

Fédérale de Lausanne) described that the AMPK signalling pathway connects to activation of SIRT1 by altering NAD⁺ levels in the cell. AMPK activators induce SIRT1-dependent deacetylation of PGC1 α , resulting in the activation of mitochondrial biogenesis pathways in muscle. David Sinclair (Harvard Medical School) suggested that SIRT1 could act as a stress sensor under conditions of DNA damage in the mouse, similarly to its role in budding yeast, and proposed a role for SIRT1 in promoting DNA repair and cell survival in a mouse model of genomic instability. Similarly, Maria Blasco (CNIO, Madrid) showed that mice carrying an additional copy of SIRT1 have lengthened telomeres and altered recombination frequencies at both telomeric and centromeric regions. Further analysing these mice, Serrano reported that—as occurs after treatment with SIRT1-activating compounds—mice overexpressing SIRT1 showed reduced rates of metabolic disease when placed on a high-fat diet and also had a lower incidence of age-associated cancer.

Stress signalling and autophagy

Several speakers discussed the connections between TOR signalling and p53. George Thomas (Genome Research Institute, Cincinnati) described a proliferation block in livers from mice lacking the ribosomal S6 protein, which is a key substrate of S6K1—a target of mTORC1. A lack of S6 results in the upregulation of p53 in a rpl11-dependent manner. In a related talk, Yanping Zhang (U. North Carolina, Chapel Hill) further detailed a ribosomal biogenesis checkpoint that signals to p53 through ribosomal protein binding to MDM2, a p53 negative regulator. Conversely, activation of p53 has been reported to lead to suppression of mTOR signalling. Michael Karin (U. California, San Diego) discussed the sestrins, another set of stress-induced signalling molecules that seemingly activate AMPK, thereby suppressing mTORC1 signalling. Different sestrin family members are transcriptional targets of p53 and FOXO, and thus suppress TOR signalling after prolonged stress stimuli. Tak Mak (Campbell Family Institute for Breast Cancer Research, Toronto) spoke of two genes originally isolated in connection with familial Parkinson disease, which connect to these same pathways governing cell survival and metabolism. The Pten-induced kinase *PINK1*—also known as *PARK6*—is

a FOXO target gene induced in response to oxidative stress. Similarly, DJ1—also known as *PARK7*—is an upstream modulator of AMPK and mTORC1 signalling that also controls HIF1 α -dependent glucose metabolism and the NRF2-dependent antioxidant response. Rafael de Cabo (Gerontology Research Center, Baltimore) showed that NRF2-dependent control of the antioxidant response is crucial in the ability of calorie-restricted mice to reduce tumour burden in models of chemical carcinogenesis. Interestingly, NRF2 knockout mice did not show reduced tumour rates as compared with wild-type controls when placed on a calorie-restricted diet, yet still retained the benefit of calorie restriction on insulin resistance and overall lifespan.

In response to alterations in mitogenic signalling pathways, cells alter their transcriptional programmes to re-engineer their metabolic consumption

The relationship between protein misfolding and endoplasmic reticulum (ER) stress was the focus of a talk from Randall Kaufman (U. Michigan Medical School). The control of translational initiation by the ER-stress-activated kinase PERK results in altered translation of the ATF4 transcription factor, promoting protein folding and antioxidant responses. The inability to signal to ATF4 or its target gene *CHOP*, results in apoptosis and various metabolic defects in different target tissues. ER stress can modulate autophagy, and one mechanism was discussed by Adi Kimchi (Weizmann Institute of Science). ER stress results in the upregulation of the death-associated protein (DAP) kinase, which phosphorylates and activates beclin as a mechanism by which to coordinate autophagic and apoptotic pathways. The p62 SQSTM1 protein, which is a common marker of autophagic response, was discussed by both Jorge Moscat (U. Cincinnati) and Eileen White (Rutgers U.). Moscat discussed both metabolic and cancer phenotypes in mice lacking p62 or its binding partners, the atypical PKC kinases. Loss of p62 or PKC ζ in mice led to increased adipogenesis, and the loss of p62 or PKC α or PKC ζ diminished K-ras-dependent tumours. The role of autophagy in promoting cell survival in metabolically stressed tumour cells was also discussed by White. As autophagy components, including

beclin (ATG6), can act as tumour suppressors, the cell survival benefit that activation of autophagy confers on stressed tumour cells seems paradoxical. Understanding the context in which autophagy is tumour-suppressive or tumour-promoting is crucial, as many current cancer therapeutics activate autophagy.

Enhancing the adaptive immune response to recognize tumour cells that are killed by chemotherapeutics was the focus Guido Kroemer (Institut de Cancérologie Gustave Roussy, Villejuif, France). The release of ATP from dying tumour cells activates the P2X7 purinergic receptor on dendritic cells, triggering caspase 1 activation and the release of interleukin (IL)-1 β and thereby stimulating the immune response. Perfectly rounding off this meeting that linked cancer to metabolic disease, Karin discussed the connection between the inflammatory release of cytokines—including IL-6 and TNF—and an increased risk of cancer in individuals with metabolic disease. Hepatocellular carcinoma (HCC) is a common form of liver cancer, and its incidence is increased four-fold in men with a high body mass index. Karin's group showed that obesity increases carcinogen-induced HCC and tumour cell proliferation, effects that are attenuated in mice lacking IL-6 or TNFR1. IL-6 seems to be a common mediator of the observed effects, perhaps by promoting hepatic steatosis and activation of the pro-growth transcription factor STAT3, although its possible impact on the TOR and AMPK pathways were mentioned as areas of future investigation in this metabolic-disease-induced tumour setting.

The field of cancer and metabolism is at a crossroads as many of the signalling pathways that coordinate nutrient uptake and catabolism with cell growth and the cell cycle machinery have begun to be identified. Skeletal outlines of the main signal transduction pathways involved and how they intersect can be made, but many details and pieces of the puzzle remain unknown in this re-emerging area at the interface of cancer and metabolism, guaranteeing lively progress for years to come.

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