Meeting Report

Metabolism and Cancer in La Jolla

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Introduction

Studies from a number of laboratories in the past decade have revealed that the central pathways deregulated in cancer often serve to coordinately regulate both classic oncogenic signaling pathways controlling cell proliferation and cell survival with pathways controlling cell metabolism. The emergence of these findings, suggesting a direct control of cellular metabolism by tumorigenic pathways, has helped fuel explosive growth in this area of cancer research. Paralleling the emergence of targeted therapeutics in cancer treatment, a variety of experimental approaches has led to the conclusion that tumors bearing some specific genetic alterations may lead them to be uniquely sensitive to agents deregulating their cellular metabolism. Given this resurgence of research at these crossroads, Metabolism & Cancer was a special conference in Cancer Research held by the AACR from September 13 to 16, 2009 in La Jolla, California.

Metabolic Requirements for Cancer Cells

Dr. Otto Warburg first described, more than 80 years ago, that a fundamental biochemical difference between tumor cells and their normal counterparts was that tumor cells rely on aerobic glycolysis for ATP generation, unlike most normal differentiated cells of the body, which use mitochondrial oxidative phosphorylation. As glycolysis is an inefficient method to produce ATP compared with oxidative phosphorylation, it remained a quandary why tumor cells might favor that mechanism. Keynote speakers Dr. Craig Thompson (University of Pennsylvania, Philadelphia, PA) and Dr. Lewis Cantley (Harvard Medical School, Boston, MA) set the stage for the meeting by providing an overview of our current understanding of the links between mammalian growth control and metabolic control. Unlike unicellular organisms, which simply upregulate operons of genes containing surface transporters and pathway components in response to the sensing of extracellular metabolites, in most cells multicellular organisms are in a constant supply of nutrients from the bloodstream. The discovery that mutations in tumor suppressors and oncogenes that promote cell survival and proliferation also stimulate nutrient uptake and alter cellular metabolism reinforces the concept that these processes are biochemically and molecularly integrated. Dr. Cantley also emphasized that, unlike glycolysis and the pentose shunt, oxidative phosphorylation cannot produce any NADPH or acetyl-coA, both of which are needed in abundance to produce phospholipids in proliferating cells. Thus the requirements for metabolic intermediates uniquely produced during glycolysis may underlie the favorable advantage it bestows on cancer cells. The PI3-kinase/mammalian target of rapamycin (mTOR) pathway and the myc pathway are centrally involved in the reprogramming of glucose and glutamine metabolism to fuel cell growth. Therefore, tumor cells bearing genetic alterations in components of these pathways become addicted to glucose and may show enhanced sensitivity to agents disrupting their energy metabolism. Whether metabolic disturbances can also explain higher rates of mutation in tumor cells remains an interesting area to investigate. Moreover, whether agents targeting key metabolic regulators—such as ATP citrate lyase, the DNA repair enzyme PARP, which requires NAD+, or the M2 splice isoform of pyruvate kinase, which the Cantley laboratory showed is critical for the proliferation of tumor cells—will be beneficial in the clinical setting remains an interesting area of ongoing investigation.

Control of Growth and Metabolism

Cells possess the capacity to sense and integrate diverse environmental signals, such as the availability of nutrients, oxygen, growth factors, and cytokines, and activity of cell adhesion molecules. However, the mechanisms underlying how eukaryotic cells integrate multiple signals to alter metabolism when faced with unfavorable conditions are less well understood. Several speakers discussed advances in our understanding of pathways that promote growth and are deregulated in many common cancers. The multiprotein mTORC1 protein kinase complex is a central component of pathways that promote growth in response to abundant levels of insulin, glucose, and amino acids. Dr. David Sabatini (Massachusetts Institute of Technology, Cambridge, MA) described how Rag proteins, a family of four related guanosine triphosphatases (GTPase), interact with mTORC1 in response to increased amino acid supplies and are necessary for mTORC1 activation by amino acids. These Rag proteins

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do not directly stimulate mTORC1 kinase activity, but promote the intracellular localization of mTORC1 to a Rab7$^+$ compartment that also contains its activator Rheb. This finding explains why other activators of Rheb, like insulin and glucose, do not stimulate mTORC1 when cells are starved for amino acids. It seems likely that Rag function could be deregulated in a variety of human tumors, which will be an important question to investigate in the future. Consistent with these findings, Rory Flinn, from Dr. Jonathan Backer's laboratory (Albert Einstein College of Medicine, Bronx, NY), presented a short talk showing that blocking the early-to-late endosome conversion step using activated Rab5 alleles or loss of the Rab7 GEF hVps39 prevents mTOR from interacting with Rheb.

TSC1 and *TSC2* are tumor suppressor genes mutated in the tumor syndrome Tuberous Sclerosis Complex. Their gene products form a heterodimer that is a critical negative regulator of mTORC1, through the GTPase-activating protein activity of TSC2 toward Rheb. Because mTORC1 activity controls a variety of anabolic processes to promote cell growth, it is highly sensitive to alterations in the extracellular environment. As such, the TSC1/TSC2 complex senses many diverse extracellular signals to properly modulate mTORC1 activity. Dr. Brendan Manning (Harvard Medical School) described recent work with TSC-deficient mouse embryo fibroblasts discerning the transcriptional output of mTORC1 activity. He has determined that the expression of hundreds of genes change in the face of TSC1-deficiency and in an mTORC1-dependent manner (i.e., they are rapamycin sensitive). Most of the factors that change in TSC-deficient cells are those that participate in critical metabolic pathways, such as glycolysis, the pentose phosphate pathway, lipid-sterol biosynthesis, and the integrated stress response.

Dr. Celeste Simon (University of Pennsylvania) focused on recent work involving a novel tumor suppressor gene known as *Birt-Hogg-Dube* (BHD). In multiple species, the pathologies of BHD are reminiscent of Tuberous Sclerosis Complex. Working with BHD-deficient mice and cells, the Simon laboratory has shown that BHD functions as a tumor suppressor by promoting the expression of the proapoptotic factor Bim downstream of TGFβ signaling. Of note, BHD-deficient tumors exhibit decreased Bim protein accumulation relative to adjacent control tissues.

The key best-studied targets of mTORC1 kinase activity are the kinase S6K1 and the translational inhibitor 4EBP1, which blocks eIF4E. A short talk by Anne Marie Kenney (Memorial Sloan-Kettering Cancer Center) described how Sonic Hedgehog (SHH) signaling in cerebellular granule neuron precursors promotes cell growth and proliferation in a manner dependent on mTORC1. Surprisingly, there was no increase in S6K1 signaling in these cells, unlike effects on eIF4E stimulation. Further examination revealed evidence of crosstalk and feedback between these two mTORC1 effector pathways, which may be regulated at least in part via SHH-mediated increases in a subunit of the PP2A protein phosphatase.

Dr. Almut Schulze (London Research Institute) described downstream targets of PI3K and mTOR signaling in the control of lipid synthesis. The sterol regulatory element binding protein (SREBP) family consists of three closely related members: SREBP1a, SREBP1c, and SREBP2. They mediate the effect of sterol on the expression of enzymes involved in lipid and cholesterol homeostasis. SREBP1 preferentially regulates genes involved in fatty acid biosynthesis. In contrast, SREBP2 regulates genes within the cholesterol pathway. It has been established that AKT induces the expression of a number of lipogenic genes, such as ATP citrate lyase and fatty acid synthase. Dr. Schulze has convincingly shown that SREBP- and AKT-dependent induction of lipid biosynthesis requires mTORC1 activity. Given that mTORC1 also promotes mRNA translation, the PI3K/AKT/mTORC1 pathway regulates protein and lipid biosynthesis in an orchestrated fashion. Moreover, both processes are required for cell growth. Because of the critical dependence of mutated cancer cells on these anabolic pathways, they may be "less metabolically flexible" and susceptible to targeted therapies that disrupt these processes.

Evelien Rysman from the Swinnen Laboratory (KU Leuven, Belgium) gave a short talk on the crosstalk between SREBP1 and *de novo* lipogenesis within primary cilia, cellular structures that play key roles in development through modulation of Wnt and Hedgehog signaling pathways.

Another central regulator of cellular metabolism is the AMP-activated protein kinase (AMPK). AMPK is activated under conditions of metabolic stress resulting in low intracellular ATP, such as when nutrients are low or following a number of pathological stresses. The kinase responsible for phosphorylating AMPK following metabolic stress is encoded by the LKB1 tumor suppressor, providing a direct connection between cell metabolism and tumorigenesis. However, in addition to AMPK, LKB1 also directly phosphorylates the activation loop threonine of 12 additional kinases all related to AMPK, drawing into question whether AMPK is the key target of LKB1 in its tumor suppressor function. Moreover, AMPK can also be phosphorylated by CAMKK2, in a manner dependent on increases in intracellular calcium. On the basis of the fact that CAMKK2 can serve as an alternative kinase for AMPK, Grahame Hardie (University of Dundee, Scotland, United Kingdom) examined whether CAMKK2 could also suppress cell growth via effects on AMPK. Recent evidence suggests that AMPK may be more widely downregulated in a number of cancers than previously thought, as phosphorylation of its downstream substrate ACC was attenuated nearly universally in breast cancer. Alternative mechanisms for suppressing AMPK besides mutation of LKB1 were discussed, including inhibitory phosphorylations on LKB1, and potential overexpression of AMPK gamma subunits in tumor cells acting as dominant negatives.

How AMPK controls cell growth was the primary focus of a talk by Dr. Reuben Shaw (Salk Institute, La Jolla, CA), which focused on suppression of the aforementioned mTORC1 signaling pathway. AMPK suppresses mTORC1 by direct phosphorylation of both the TSC2 tumor suppressor and the mTORC1 scaffolding subunit raptor, resulting in a metabolic checkpoint on cell cycle progression. As LKB1-deficient tumors exhibit elevated mTORC1 signaling, this

suggested rapamycin may be a potential therapeutic. In a mouse model of Peutz-Jeghers syndrome, LKB1$^\pm$ mice develop gastrointestinal hamartomas similar to their human counterparts, and treatment of these mice with rapamycin resulted in dramatic suppression of their tumor burden. Hypoxia inducible factor (HIF)-1α was identified as a target of mTORC1 in these tumors, and its targets involved in glucose metabolism, including the GLUT1 glucose transporter and Hexokinase II, were upregulated. Consistent with increased glucose uptake and glycolysis, hamartomas in the LKB1$^\pm$ mice showed marked accumulation of ^{18}F-deoxyglucose (FDG), which was reversed by rapamycin, as visualized by imaging wild-type and LKB1$^\pm$ mice with positron emission tomography (FDG-PET).

In addition to the treatment of LKB1-deficient tumors with mTORC1 inhibitors, a related question is whether agents that activate AMPK will be clinically useful as anticancer therapeutics due to their ability to suppress mTOR and other growth-promoting pathways. Scott Rothbart from the laboratory of Dr. Richard Moran (Virginia Commonwealth University, Richmond, VA) presented results that the widely used antifolate cancer drug pemetrexed, in addition to targeting thymidine synthase, acts on the enzyme aminoimidazolecarboxamide ribonucleotide formyltransferase (AICART), which is a folate-dependent enzyme in de novo purine synthesis. Inhibition of AICART results in a buildup of ZMP in pemetrexed-treated cells, which mimics AMP, activating AMPK and resulting in mTOR suppression. It was hypothesized that this effect of pemetrexed on AMPK and mTOR may explain the activity of pemetrexed in lung cancers, which is unusual for antifolate compounds. A physiological inducer of AMPK activity is the adipose-produced cytokine adiponectin. Preet Dhillon (Harvard School of Public Health) gave a short talk on the association of different polymorphisms in the adiponectin gene and the genes of the two adiponectin receptors (AdipoR1, R2) in prostate cancer susceptibility, as adiponectin levels have been shown to correlate with cancer risk in previous epidemiology studies.

Transcriptional Control of Metabolism

The survival of multicellular organisms is critically dependent upon O_2 use, which, as a substrate for cytochrome c oxidase, allows the generation of intracellular ATP. The tumor microenvironment is characterized by regions of fluctuating O_2 and nutrient depletion. Transcription factors, called HIFs, play an essential role in O_2 homeostasis. Of note, HIF controls the expression of hundreds of genes in response to O_2 limitation. Dr. Gregg Semenza (Johns Hopkins University, Baltimore, MD) described how under hypoxic conditions ATP production by mitochondria is accompanied by the generation of reactive oxygen species (ROS) because of premature transfer of electrons at complex I or complex III of the electron transport chain. Cells must adapt their metabolism to survive chronic hypoxia. One adaptation regulated by HIF-1 is a subunit switch in cytochrome oxidase (electron transport complex IV), which is orchestrated by HIF-1 to optimize respiration under hypoxic conditions. HIF-1 also prevents excessive ROS production in hypoxic cells by regulating mitochondrial activity in numerous ways. For example, HIF-1 induces the expression of pyruvate dehydrogenase kinase 1, which phosphorylates and inactivates pyruvate dehydrogenase, the enzyme that converts pyruvate to acetyl coenzyme A for entry into the TCA cycle. Moreover, HIF-1 negatively regulates mitochondrial biogenesis and O_2 consumption by inhibiting c-Myc activity. Given the essential role HIF-1 plays in a broad spectrum of malignancies, Dr. Semenza's laboratory is now identifying small molecule inhibitors of this pathway. Many of these seem to be DNA-intercalating reagents, which inhibit HIF/DNA interactions. One ripe area for development includes inhibitors of HIF-1α/HIF-2α dimerization with their common cofactor ARNT.

Dr. Randy Johnson (University of California, San Diego, La Jolla, CA) has been studying the normoxic regulation of HIF. One important regulatory protein is "factoring inhibiting HIF" (FIH), an asparagine hydroxylase, which is ubiquitously expressed. FIH-deficient animals display increased metabolic rates, breathing frequency, and lung tidal volume. Marian Koritzinsky (University of Toronto, Toronto, Canada) provided some novel data describing how the unfolded protein response (UPR) protects cells during hypoxia through preservation of autophagic capacity. Finally, Dr. Mark Van Gilst (Fred Hutchinson Cancer Research Center, Seattle, WA) described how hypoxic exposure stimulates fatty acid synthesis via inhibition of oxidative phosphorylation in Caenorhabditis elegans, as a means of maintaining redox homeostasis.

The most commonly mutated tumor suppressor gene in human cancer is the p53 transcription factor. Though first appreciated to control cell cycle progression and apoptosis through expression of distinct groups of downstream transcriptional targets, in the past 5 years, it has become apparent that p53 also contributes to the control of metabolism through additional transcriptional targets. Karen Vousden (Beatson Institute, Glasgow, Scotland, United Kingdom) described studies of p53 regulation of TIGAR, a potential antagonist of glycolysis by acting as a dominant negative PFK2 molecule. p53 also inhibits glucose uptake and promotes oxidative phosphorylation, though all of the mechanistic details involved remain to be fully elucidated. Another transcription factor also tied to upregulation of glucose transporters is nuclear factor κ-B (NF-κB), a well-established regulator of inflammation and cell survival in both hematopoietic and epithelial lineages. Exploiting a well-studied K-ras–dependent model of non–small cell lung carcinoma, Dr. Etienne Meyhaus from Dr. Tyler Jacks' laboratory (Massachusetts Institute of Technology, Cambridge, MA) examined how p53 loss synergizes with K-ras to regulate NF-κB signaling. Creating an inducible repressor of NF-κB, they showed the requirement for NF-κB signaling in lung tumor development, which is dependent on p53 status.

Another transcription factor family that is central to both cell growth and metabolic control is the Myc family, typified by the c-Myc oncogene, but also comprising a family of basic helix-loop-helix transcription factors, which bind DNA as

heterodimers and can act as both transcriptional activators and repressors. Donald Ayer (University of Utah, Salt Lake City, UT) discussed one of the Myc family members that serves as a transcriptional activator of metabolic genes, the MondoA protein, which heterodimerizes with Mlx. MondoA is a close relative of ChREBP, an established promoter of lipogenesis, which is apparently exclusively expressed in liver. MondoA, in contrast, seems to be widely expressed across adult hematopoietic, epithelial, and mesenchymal lineages and is found to localize subcellularly to the outside of the mitochondria. MondoA localization is altered by 2-deoxyglucose, and MondoA-induction of target genes is also dependent on glutamine, making it a unique responder to multiple metabolic inputs.

The question of how glucose and glutamine levels themselves are coordinately regulated by oncogenes was the focus of a short talk by Dr. Mariia Yuneva from Dr. Michael Bishop's laboratory (University of California, San Francisco, San Francisco, CA). Exploiting a series of inducible oncogenes (Myc, Ras, Met) in mouse models of hepatocellular carcinoma, and doing both mRNA and metabolic profiling, revealed a number of common and unique signatures across these oncogenes, which may be useful for future therapeutic interventions or diagnostic biomarkers. Dr. Jihye Yun from Dr. Bert Vogelstein's laboratory (Johns Hopkins University, Baltimore, MD) described how glucose deprivation contributes to the development of K-Ras pathway mutations in tumors. Global gene expression analysis using paired isogenic colorectal cancer cell lines, differing in the mutational status of *KRAS* or *BRAF* genes, indicated that *GLUT1* is consistently upregulated in cells with these mutations. These cells exhibit enhanced glucose uptake and lactate production and survive glucose starvation. When wild type cells are subjected to glucose deprivation, those cells that survive express high levels of *GLUT1* and acquire *KRAS* mutations. In contrast, low O_2 does not provide a selective growth advantage to cells with *KRAS* or *BRAF* mutations. The conclusion from these studies is that nutrient availability in the tumor microenvironment can be a major force in promoting mutations in key oncogenes.

Nuclear receptors are a large family of transcriptional factors that control metabolic physiology in a wide variety of tissues. Many connections between nuclear receptors and cancer have been made over the years including studies on thyroid hormone, the peroxisome proliferation-activated receptors (PPAR), and the prominent role of estrogen and androgen receptors in hormone-sensitive tissue tumors. How nuclear receptor-mediated control of metabolism is tied to classic growth-promoting and growth-suppressive signaling pathways is an area currently under significant investigation. Ron Evans (Salk Institute, La Jolla, CA) described recent studies showing that AMPK is a key activator of PPARδ-dependent transcription and the two play important roles in muscle in promoting endurance following exercise. Strikingly, the AMP-mimetic AICAR was able to promote PPARδ-dependent increases in an endurance-associated gene signature and AICAR as a single agent was capable of promoting endurance in sedentary animals. Additional

studies suggested another mechanism through which AMPK regulates metabolic transcription. AMPK was found to directly phosphorylate the Cry1 transcriptional corepressor, a core component of the circadian clock, which controls a wide variety of physiological processes through effects on transcription. In mammals, clocks in peripheral tissues are more influenced by feeding than by sunlight and activation of AMPK was shown to be a potential mediator of that effect. Activated AMPK directly phosphorylates Cry1 and induces its association with the F-box protein Fbxl3, which targets Cry1 for degradation, functionally altering the circadian clock.

The estrogen receptor-related proteins are a subfamily of three nuclear receptors that regulates mitochondrial biogenesis, in some contexts in conjunction with the PGC-1α and beta transcriptional co-activators. Anastasia Kralli (The Scripps Research Institute, La Jolla, CA) described how mice deficient in ERRα show loss of mitochondrial mass and functional defects in brown fat, muscle, and macrophages. An examination of whether these proteins may play a role in cancer or cell growth was examined in breast cancer cell lines and revealed a potential requirement for some family members in aspects of tumorigenesis in addition to their established roles in oxidative metabolism.

Another group of nuclear receptors recently connected to growth control are the liver X receptors (LXR), which are cholesterol sensors whose endogenous ligands are oxysterols and intermediate metabolites in cholesterol synthesis. Although LXRα is predominantly found in liver, adipose, and macrophages, LXRβ is more ubiquitous in its expression. Peter Tontonoz (University of California, Los Angeles) reported that LXRβ null mice exhibit profound splenomegaly and lymphocyte expansion, which prompted an examination of LXRβ function in proliferation. During normal T-cell activation, SREBP target genes were upregulated, whereas LXR targets were downregulated. Forced activation of LXR can also prevent cell cycle progression. Further studies revealed that LXR activation reduced LDLR protein levels. A RING domain-containing protein named Idol, which serves to degrade LDLR, was identified as an LXR target gene and was further shown to regulate LDL in circulating blood plasma. The control of cholesterol homeostasis in all cells and its relationship to growth control is a very interesting emerging area of study.

Another key class of metabolically targeted transcriptional regulators is the class III family of histone deacetylases, known as sirtuins. Leonard Guarente (Massachusetts Institute of Technology, Cambridge, MA) discussed how distinct members of the sirtuin family influence different aspects of metabolism and growth control, in part through their differing subcellular localization. Sirt1, 6, and 7 are all predominantly nuclear, though Sirt1 has also been reported to be found in mitochondria whereas Sirt3, 4, and 5 are all predominantly mitochondrial. Interestingly, roles for Sirt1 in both tumor progression and tumor suppression can be found in opposing mouse models of Sirt expression. Thus the role of Sirt1 in cell survival and metabolism may likely be cell-type specific.

Mitochondria, Glucose Uptake, and Glycolysis

As proteomic efforts have started cataloging all the components of both yeast and mammalian mitochondria, it has become apparent that as many as 25 to 40% of mammalian mitochondrial proteins are poorly or incompletely characterized. Jared Rutter (University of Utah, Salt Lake City, UT) described efforts in his laboratory to study some of the highly conserved components of the mitochondria, which led them to focus on a highly conserved gene they named *SDH5*. *SDH5* was found to be required for respiration in yeast through its regulation of the succinate dehydrogenase complex (SDH). Notably, hereditary forms of paraganglioma, a neuroendocrine tumor, have been identified as being due to mutations in other core subunits of the SDH complex (SDHB, SDHC, and SDHC), and human SDH5 is mutated in some kindreds with paraganglioma, whose tumor suppressor had not yet been identified. These findings reinforce that genetic perturbations in core metabolic complexes can in fact lead to some forms of human cancer.

Similarly, it is now clear that a majority of low-grade gliomas (the most common type of human brain tumor) exhibit mutations in one of two $NADP^+$-dependent isocitrate dehydrogenase enzymes (IDH1 and IDH2). Dr. Kun-Liang Guan (University of California, San Diego) suggested that tumor-derived IDH1 mutations impair the enzymes' affinity for its substrate and inhibit wild-type enzymatic activity and formation of α-ketoglutarate, the enzyme product. Interestingly, α-ketoglutarate is necessary for destabilizing HIF-1α, which may contribute to glioma development. Subsequent studies have also suggested a gain-of-function role for IDH1 and IDH2 mutations. Dr. Guan also discussed recent data on proteomic identification of a large number of acetylated proteins. Many of these are enriched in metabolic pathways, such as the TCA cycle, fatty acid synthesis, the urea cycle, gluconeogenesis, and glycolysis.

In addition to central roles in energy homeostasis, mitochondria also serve as central players in apoptosis, and the permeabilization of the outer mitochondrial membrane by pro-apoptotic Bcl-2 family members Bax and Bak is a key event in this process. Doug Green (St. Jude's Children Hospital) described how the ability of Bax and Bak to promote this can be shown using mitochondrial preparations and peptides to facilitate oligomerization with other Bcl-2 family members and other protein interactors. Strikingly, highly purified preps of mitochondria lacking endoplasmic reticulum contamination lose the ability to promote activation of Bax/Bak, and this was shown to depend on one or more neutral sphingomyelinases (SMase), which are enzymes that hydrolyze sphingomyelin to ceramide. Through a variety of experimental perturbations, sphingosine-1-P was shown to be critical in this process. These findings suggest that the endoplasmic reticulum is more critical for some mitochondrial functions than previously thought and that lipid-protein interactions play an unexpected important role in mitochondrial-based apoptosis.

In contrast to apoptosis, which serves to eliminate damaged cells from the body, autophagy is the process of self-cannibalism, which cells undergo to attempt to maintain survival under nutrient-poor or other stress-induced conditions. A conserved autophagy regulator beclin, which is also a Bcl-2 interacting protein, was shown to directly connect autophagy to cancer several years ago when it was found to be a human tumor suppressor gene. As Vassiliki Karantza (Cancer Institute of New Jersey) described, this led to an interesting paradoxical question of how loss of survival mechanism would lead to cancer because increased survival by Bcl-2 family members or mitogenic signaling, for example, is often thought to allow mutant cancer cells to survive when they otherwise would not, promoting tumorigenesis. Two mechanisms by which autophagy can suppress cancer risk were discussed: non-cell autonomous suppression of necrosis and inflammation and cell-autonomous suppression of genomic instability. Their studies suggest that high basal levels of autophagy may be a very common feature of cancer cells. Importantly, autophagy-competent tumor cells become addicted to autophagy as a survival mechanism, and thus pharmacological autophagy inhibition may act as a chemosensitizer. Conversely, in "autophagy incompetent" tumor cells, autophagy-inducing stimuli may drive tumor cells to undergo apoptosis because they are autophagy defective.

Regulation of mitochondrial mass is another key adaptive response to nutrient deprivation and hypoxia. Interestingly, HIF-mediated control of mitochondrial autophagy (mitophagy) has been shown to involve the BH3 domain-containing proteins bNIP3 and bNIP3L/Nix. Danielle Glick from Dr. Kaye Macleod's laboratory (University of Chicago) examined the role of bNIP3 in growth and metabolic control in liver. bNIP3-deficient hepatocytes displayed phenotypes consistent with a central role for this protein in mitophagy.

As stated above, mitochondria produce increased ROS under conditions of energetic imbalance and O_2 limitation. Both Dr. Tak Mak (University of Toronto) and Dr. Navdeep Chandel (Northwestern University) described how graded levels of ROS result in a variety of cellular responses. For example, low ROS levels can engage cell proliferation (via the Ras/MEK/ERK pathway), intermediate levels promote metabolic adaptations through HIF, and high levels can result in senescence and/or cell death through p53. Cell toxicity is also derived from the damaging effects of high ROS levels on DNA, protein, and lipids. A variety of intracellular metabolites modulate ROS accumulation, such as NADPH, glutathione, and peroxiredoxin. DJ-1 (also called CAP1 or PARK7) when overexpressed confers resistance to oxidative stress, and DJ-1 inhibition enhances cytotoxicity mediated by hydrogen peroxide and MPTP. Therefore, DJ-1 is considered to be an antioxidant protein. Dr. Mak described how DJ-1 is critical for AKT and mTORC1 activity that sustain HIF-1α accumulation. Moreover, DJ-1 regulates the activity of the AMPK metabolic sensor, especially during hypoxia. Collectively, DJ-1 is an upstream activator of HIF function through its regulatory effects on mTORC1 and also a key player in promoting hypoxic adaptations via AMPK. Dr. Chandel carried on the discussion of mitochondria as signaling organelles. He has previously shown that ROS promote HIF, AMPK, and

MAPK activity. He is currently probing a specific role for mitochondria in tumor progression by inhibiting mitochondrial function in a K-Ras driven model of lung tumorigenesis.

Dr. Nicolas Denko (Stanford University, Palo Alto, CA) described a screen for genetic elements that accelerate the growth of pancreatic tumors and identified the mitochondrial ribosomal protein L28. L28 inhibition in these cells decreased mitochondrial activity and increased glycolysis, but also decreased cellular growth *in vitro*. However, L28 inhibition caused decreased mitochondrial function, compensatory increases in glycolysis, and accelerated pancreatic tumor cell growth *in vivo*. These findings were interpreted to show that nononcogenic genetic changes altering mitochondrial metabolism can regulate tumor growth through modulation of O_2 consumption, which seems to be a rate-limiting substrate for tumor proliferation. These findings emphasize how the Warburg effect must be studied in bona fide tumors, in which nutrient and O_2 availability are limited by blood supply (conditions difficult to model *in vitro*).

Dr. Magdalena Szelag from Dr. Andrew Larner's laboratory (Virginia Commonwealth University, Richmond, VA) described novel observations indicating that JAK-STAT signaling impacts mitochondrial activity. Their recent studies suggest a novel role for Stat3 and Stat1 not only in nuclear control of transcription, but also that these transcription factors can be found in the mitochondria in a variety of cultured cells and tissues. Intriguing new data connecting the role of STATs to regulation of mitochondrial function were presented.

The endoplasmic reticulum is a specialized organelle for chaperone-assisted protein folding and post-translational protein modification. Disruption of endoplasmic reticulum homeostasis via increased biosynthetic load ("proteotoxicity") results in the accumulation of unfolded proteins and activation of the UPR. This results in the activation of IRE1, ATF6, and PERK. Dr. Randy Kaufman (University of Michigan, Ann Arbor, MI) described how PERK phosphorylation of eIF2α and translation attenuation prevents oxidative stress in pancreatic β cells. Absence of this adaptive pathway causes a severe diabetic phenotype and β cell apoptosis. In mice with a disrupted UPR, glucose intolerance and β-cell death is attenuated by antioxidants. The conclusion is that eIF2α phosphorylation limits mRNA translation, prevents oxidative stress, and optimizes endoplasmic reticulum protein folding to support insulin production. Dr. Jeffrey Rathmell (Duke University, Durham, NC) has been studying how increased glucose metabolism can inhibit cell death and deter-

mined that Bcl-2 family protein regulation is critical. Increased glucose metabolism results in GSK-3 inhibition and Mcl1 stabilization to prevent apoptosis. Decreased glucose metabolism leads to the induction of pro-apoptotic Bcl-2 family members, such as Puma and Bim. The overall goal is to further elucidate connections between Bcl-2 family proteins and cellular metabolism for exploitation in the development of drugs against a variety of cancers. Dr. Aimee Edinger (University of California, Irvine, Irvine, CA) described a novel approach involving the inhibition of nutrient transporter expression. Metabolic changes associated with malignant transformation render cancer cells highly dependent on a high rate of nutrient influx. This strict requirement for nutrient uptake led her to study sphingolipid analogs and how they control nutrient transporters proteins in mammalian cells and whether they may hold therapeutic potential. In closing the program, Dr. Alan Diehl (University of Pennsylvania) returned the discussion to the effects of PERK on tumor progression using MMTV-Neu-dependent mammary tumors in mice. He has shown that PERK inhibition results in decreased tumor growth. Furthermore, PERK inhibition compromises cancer cell redox homeostasis resulting in DNA damage, DNA damage checkpoint activation, and p53-independent G2/M growth arrest. These results make PERK a highly attractive target for the development of antineoplastic drugs.

Summary and Concluding Statement

As the molecular details connecting cancer and growth control to metabolism continue to be unraveled, novel cancer targets will be identified that may be therapeutically exploited to target the unique metabolic state of tumor cells. Despite a century of studies on the biochemical pathways controlling metabolism in cells and several decades uncovering the pathways underlying the development of human cancer, only in the past decade has the intimate molecular interconnection between these two become uncovered. It is safe to assume there are a number of fundamental insights into how proliferation and metabolism are coupled that are yet to come.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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