Twenty-fifth Annual Pezcoller Symposium: Metabolism and Tumorigenesis

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Abstract

Choking cancer via inhibition of metabolic enzymes essential for tumor but dispensable in normal tissues was discussed as was the altered metabolism in cancer cells related to tumor suppressor protein (pVHL) function, the histone acetylation dependence upon glucose, the epigenomic reprogramming of acetyl CoA synthesis, the plasticity of aging mechanisms, and the metabolism orchestration in macrophage polarization. The p53 and p73 pathways role in metabolic adaptation, the effects on growth of AMP-dependent kinase, the growth regulation by the mTOR pathways, and the bioenergetics requirements of cancer cells were also discussed. A novel computational model of personalized metabolic changes in cancer was outlined with applications in patients with breast cancer. Imaging metabolic characteristics of tumors by MRI and 13C-nuclear magnetic resonance was described. The cancer metabolism regulation related to O-linked β-N-acetylgalactosamine was described. DNA hypermethylation and impaired hematopoietic differentiation in AML after isocitrate dehydrogenase 1/2 mutation and 2-hydroxyglutarate increases were outlined. Cancer Res; 73(20); 6124–7. ©2013 AACR.

Findings Presented

Steven McKnight (University of Texas Southwestern Medical Center, Dallas, TX) discussed the possibility of choking cancer via inhibition of metabolic enzymes dispensable to normal tissues. The properties of mouse embryonic stem cells and their metabolic function during proliferation and differentiation were outlined, and their dependence upon the conversion of threonine into glycine and acetyl CoA was indicated. Selective chemical inhibitors of threonine dehydrogenase kill mouse embryonic stem cells, but not cells that do not express the enzyme. Certain solid tumors use acetate as a critical carbon source. An enzyme converting acetate into acetyl-CoA is dispensable to all tissues of adult mice, but is critical to a substantial fraction of hepatic tumors. It is hoped that potent inhibitors of this enzyme will qualify as nontoxic therapeutics for acetate-dependent human tumors.

The metabolic regulation and cancer cells

William Kaelin (Dana Farber Cancer Institute, Boston, MA) indicated that the binding of pVHL to hypoxia-inducible factor-1α (HIFα) requires HIFα to be hydroxylated by the EglN prolyl hydroxylases. The EglNs are 2-oxoglutarate–depen-

dent dioxygenases that require oxygen and iron for their function. Recently, another 2-oxoglutarate–dependent dioxygenase, the H3K4 demethylase KDM5A, was found to be a potential therapeutic target in murine cancers. Cancer-associated inactivating mutations affecting SDH and FH lead to the accumulation of succinate and fumarate, respectively, whereas gain of function isocitrate dehydrogenase (IDH) mutations lead to 2-hydroxyglutarate (2HG) accumulation. These metabolites modulate 2-oxoglutarate–dependent dioxygenases. IDH mutations have been observed in gliomas and leukemias. R2HG, in contrast with the alternative enantiomer S-2HG, potentiates, rather than inhibits, EglN and EglN loss transforms human astrocytes (1). R2HG is sufficient to reversibly promote leukemic transformation (2). It does so by inhibiting TET2, while sparing EglN activity.

Kathryn E. Wellen (University of Pennsylvania, Philadelphia, PA) discussed cancer cell metabolic reprogramming and regulation of the epigenome. Acetylation of histones is sensitive to glucose availability through ATP-citrate lyase (3), which produces acetyl-CoA from citrate. This may impact gene expression and other chromatin-dependent processes. Acetyl-CoA levels are dynamically regulated by glucose availability, and glucose-derived acetyl CoA is rapidly incorporated into acetylated histones. Oncogene-induced metabolic rewiring may induce changes in acetyl-CoA levels, which affect histone acetylation and tumor growth (4).

Anne Brunet (Stanford University, Stanford, CA) discussed metabolic regulation of aging using the C. elegans model. Aging is regulated by genetic (30%) and environmental (70%) factors. The pathway connecting insulin signaling to FoxO transcription factors plays a pivotal role in aging. Novel pathways control longevity, particularly in response to dietary restriction (5). The short-lived African N. furzeri is a new vertebrate model to explore the genetic architecture of longevity.
Alberto Mantovani (Istituto Clinico Humanitas and Milan University, Rozzano, Italy) indicated that macrophages are orchestrators of chronic inflammation. They respond to microenvironmental signals with polarized genetic and functional programs. M1 macrophages kill microorganisms and tumors. M2 cells tune inflammation and adaptive immunity, promote cell proliferation and angiogenesis, tissue remodeling and repair (6). M2-like polarization of phagocytes orchestrates the inflammation associated with established neoplasia (7).

**Metabolic pathways in cancer cells**

Karen Vousden (The Cancer Research UK Beatson Institute, Glasgow, United Kingdom) outlined the p53 role in metabolic adaptation and survival. p53-deficient cells may be more vulnerable to metabolic stress, with evidence that p53 expression helps cells survive glucose and serine starvation. Serine starvation induces de novo serine synthesis, accompanied by lower flux through glycolysis and an increase in oxidative phosphorylation. p53 is not necessary for serine synthesis activation but seems to be required for cells to undergo this metabolic adaptation and survival. p53-induced proteins limit ROS and modulate metabolism, and could contribute to tumor suppression by preventing the accumulation of stress or damage. However, the inappropriate expression of some of these p53 target genes may also support cancer progression. TIGAR is a p53-inducible protein that protects cells from death. TIGAR acts as a fructose-2,6-bisphosphatase, promoting NADPH production to restore reduced glutathione (GSH) and protecting the cell from ROS-associated apoptosis and autophagy. In mice, TIGAR plays a role in limiting ROS in proliferating adult tissue. Lack of TIGAR impedes the regeneration of damaged epithelium after irradiation, but also retards cancer development. Thus TIGAR may be a target for therapeutic intervention.

Reuben Shaw (Salk Institute for Biological Studies, LaJolla, CA) indicated that the serine/threonine kinase, LKB1, is a tumor suppressor gene mutated in the Peutz–Jeghers syndrome and in 30% of non–small cell lung cancer. A critical LKB1 substrate is the AMP-activated protein kinase (AMPK). AMPK restores metabolic homeostasis following stress. LKB1 is a unique energy-state regulator of growth and metabolic reprogramming via its effects on AMPK. Components of mTOR signaling, the autophagy pathway, and transcriptional regulators of metabolism are direct substrates of AMPK. Novel LKB1 and AMPK effectors coordinate cell growth with energy status. Metformin activates AMPK, and metformin and the related compound phenformin have anti-lung cancer activity. Notably, although cells containing an intact LKB1-AMPK pathway respond to metabolic stress by undergoing growth arrest, tumor cells lacking LKB1 continue to divide and are driven into apoptosis. Phenformin showed specific efficacy in an LKB1-mutant lung cancer mouse model, so now interest lies in whether this can be translated into the oncology clinic.

David Sabatini (Whitehead Institute, MIT, Cambridge, MA) discussed mTOR as the central component of a nutrient- and hormone-sensitive signaling pathway regulating cell growth and proliferation, playing an important role in the control of metabolism and aging. mTOR-containing protein complexes have been identified, one which regulates growth through S6K and another that regulates cell survival through Akt. GTPases have been identified that mediate amino acid binding to mTOR.

Almut Schulze (CRUK London Research Institute, London, United Kingdom) discussed how metabolic reprogramming in cancer supports cell growth and survival. Alterations in metabolic activity are an important feature of cancer cells, and many metabolic processes are regulated by oncogenic signaling pathways. Cancer cells have to balance their bioenergetic requirements with antioxidant synthesis. Disruption of this balance leads to loss of viability and may offer therapeutic opportunities. Activation of SREBP by the phosphoinositide 3-kinase pathway induces lipid synthesis and cell growth (8). Inhibition of SREBP induces endoplasmic reticulum (ER) stress-blocking protein synthesis by preventing lipid desaturation. Oleate repletion prevents ER stress in response to SREBP depletion. Moreover, a screen of 240 metabolic genes in genetically diverse breast cancer cell lines revealed the importance of pH regulation for cancer cell survival.

Grahame Hardie (University of Dundee, Dundee, United Kingdom) indicated that AMPK is a highly conserved sensor of cellular energy existing as complexes containing catalytic α subunits and regulatory β and γ subunits, and is activated by phosphorylation at a threonine residue within the α subunit. Displacement of ATP by AMP and/or ADP at the γ subunit enhances threonine phosphorylation, leading to an increase in kinase activity. The hunt for upstream kinases that phosphorylated threonine led to the identification of LKB1. Thus AMPK might mediate some of the tumor suppressor functions of LKB1. AMPK activation triggers cell-cycle arrest, inhibits the mTOR complex (mTORC)-1 pathway and other anabolic pathways required for cell growth, and opposes the reliance on glycolysis seen in most tumor cells while promoting the more energy-efficient oxidative metabolism. The presence of AMPK delays the onset of tumors or renders them less aggressive. The LKB1-AMPK pathway is a tumor suppressor and is down-regulated in many tumors and seems to protect against the development of cancer. Paradoxically, it enhances the survival of tumor cells treated with cytotoxic agents.

**Modeling Cancer Metabolism**

Massimo Loda (Dana Farber Cancer Institute, Boston, MA) outlined the prostate lipogenic phenotype and its regulation by AMPK. It is unknown whether all oncogenes harness a similar metabolic response in human tumors or whether each oncogene drives its own metabolic program. Metabolite profiling was conducted on human and murine models of prostate cancer driven by different oncogenes. An integrative analysis of the metabolomics profiles showed that some oncogenes activated in the prostate predominantly drive aerobic glycolysis, whereas others are associated with dysregulation of lipid metabolism. Importantly, prostate tumors exhibit metabolic fingerprints of their molecular phenotypes, which may have high impact on diagnostics. Targeted therapeutics with AMPK activators may be used in lipogenic tumors.
Owen Sansom (The Beatson Institute of Cancer Research, Glasgow, United Kingdom) discussed how translational elongation is limiting for tumorigenesis following Apc loss. The Apc gene is mutated in approximately 80% of colorectal cancers. It negatively regulates Wnt signaling. Loss of Apc leads to nuclear β-catenin accumulation, TCF/LEF target genes expression, and to a phenotype where enterocytes fail to differentiate, hyperproliferate, and are unable to migrate. Codelletion of c-Myc rescues the phenotypes of Apc loss. mTor, 4EEBP1, and S6 kinase phosphorylation are Myc dependent. Inhibition of mTORC1 function had marked effects on APC-deficient cells. Data were presented to suggest that rather than translational initiation, translational elongation was the crucial effector for mTORC signaling following Apc loss. Additional oncogenic mutations, common in colorectal cancers, were able to alter both the dependence upon mTORC signaling and the metabolism of Apc-deficient cells. Therefore the efficacy of mTOR inhibition in colorectal cancer is likely to be both stage (e.g., early stage) and driver mutation specific.

Gerry Melino (MRC Toxicology Unit, Leicester, United Kingdom and University Tor Vergata, Rome, Italy) indicated that TAp73-null mice show a premature spontaneous aging phenotype at 14 months of age. TAp73 protects against aging by regulating mitochondrial activity and preventing ROS accumulation. TAp73-null mice show unbalanced mitochondrial redox defenses, in part, mediated by direct transcriptional regulation of Cox6h. TAp73 also drives the expression of glutaminase type 2 and regulates the serine synthesis. TAp73-null cells show clear metabolic defects in the glutamine/serine pathway, affecting GSH and redox balance.

Eytan Ruppin (Tel-Aviv University, Tel-Aviv, Israel) discussed a novel computational approach termed PRIME (Personalized Reconstruction of METabolic models), which generates individualized genome-scale metabolic models based on molecular and phenotypic data. More than 250 personalized metabolic models for the HapMap and NCI-60 cancer cell lines successfully predicted metabolically related phenotypes including proliferation rates, gene essentiality, drug responses, metabolic biomarkers, and known selective drug treatments in cancer. PRIME-derived models of breast cancer enhanced prognosis prediction. With the NCI-60 models, a genome-scale investigation of the Warburg effect was conducted. The ratio between the production of ATP in the glycolysis and its production in OXPHOS, an index of cells “Warburgness” was strongly associated with central cancer-related features.

**Imaging and new technologies**

Kevin Brindle (University of Cambridge, Cambridge, United Kingdom) described methods for detecting early tumor responses to therapy using MRI of cell metabolism with hyperpolarized 13C-labeled metabolites. Nuclear spin hyperpolarization can increase the sensitivity of MRI by exchange of polarization.

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with a distinct evolutionary origin. *IDH* mutant gliomas produce 10- to 100-fold increased levels of 2HG. Diagnostic imaging of 2HG identified *IDH* tumor formation. Therapeutic strategies for *IDH*-mutant versus wild-type malignant gliomas are likely to be different; favorable responses of patients with *IDH*-mutant gliomas likely contribute to the better prognosis in this subgroup. Individualized clinical strategies for malignant astrocytoma may be based on *IDH* status.

**Therapeutic Opportunities**

Katherine Yen (Agios Pharmaceuticals, Cambridge, MA) discussed *IDH1* and *IDH2* genes mutations present in approximately 20% of acute myelogenous leukemia (AML) and resulting in 2HG production. In AML, *IDH1*/2-mutant displays hypermethylation and impaired hematopoietic differentiation. Erythroleukemia cell line (TF-1) transfected with either *IDH1*- or *IDH2*-mutant alleles overexpresses the mutant enzyme, has high levels of 2HG, and exhibits granulocyte macrophage colony-stimulating factor-independent growth. *IDHm* enzymes inhibit reverses hypermethylation of both histones and DNA and induces cellular differentiation in *IDHm* cell lines and primary *IDHm* AML patient samples (2, 12, 13). Inhibitors of *IDH1*/2 mutations could correct the altered gene expression patterns seen in *IDH1*/2-mutant AML.

Raymond Pagliarini (Novartis Institutes for Biomedical Research, Inc, Cambridge, MA) indicated that *IDH1* and *IDH2* mutations result in gains of enzymatic functions and overproduction of 2HG. Inhibition of *IDH*-dependent 2HG production is a strategy to treat patients bearing *IDH* mutations. Isogenic *IDH*-mutant and wild-type cells were generated. *IDH* mutations and also 2HG promote reversible epithelial–mesenchymal transition in these cells (14). *IDH*-dependent metabolic phenotypes were also shown. *IDH*-dependent glutamine contribution to AcetylCoA and citrate incorporation under hypoxic conditions was measured. In tumors with mutant *IDH1*, inhibition of mitochondrial metabolism had antitumor effects.

**Summary**

A significant benefit derived from this meeting was the unequivocal realization that molecular changes in metabolism unique for tumors provide opportunities for selective interventions. The relationships between oncogene function and metabolism were repeatedly indicated, as was the histone acetylation dependence upon glucose and the epigenomic reprogramming of acetyl CoA synthesis. The metabolic functions of AMPK and the consequences of balancing the bioenergetic requirements of cancer by antioxidants synthesis, pH regulation, and stress responses were emphasized. The role of p53 and p73 in metabolic adaptation, aging, and survival was discussed. The potential sites of intervention derived from APC loss were outlined. The *IDH* mutations and the 2HG-dependent phenotypes were repeatedly discussed. A previously characterized mechanism for the regulation of O-GlcNAc–related pathways was outlined. Novel approaches to imaging tumor metabolic characteristics and new methods for metabolic profiling were discussed. A novel computational model of personalized metabolic changes was described and its use for prognosis of patients with breast cancer indicated. Throughout the meeting, several new ideas were formulated toward identifying unique metabolic sites that could be exploited therapeutically without affecting normal tissues.

**Disclosure of Potential Conflicts of Interest**

W. Kaelin Jr is employed in Eli Lilly and Company, is a member of board of directors and a consultant/advisory board member in Agios and FibroGen. No potential conflicts of interest were disclosed by the other authors.
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