Meeting Report: Exploiting the Tumor Microenvironment for Therapeutics

Giovanni Melillo and Gregg L. Semenza

Abstract

Recent progress in understanding the role of the tumor microenvironment in cancer progression was the subject of the 2nd International Tumor Metabolism Summit entitled "Exploiting the Tumor Microenvironment for Therapeutics," a meeting held at Palazzo Ducale in Genoa, Italy, October 7 to 8, 2005. One of the major conceptual advances in oncology over the last decade has been the appreciation that all major aspects of cancer biology are influenced by the tumor microenvironment. Two important means by which cancer cells adapt to their microenvironment are by reprogramming cellular glucose/energy metabolism to use pathways that generate ATP in the absence of O2 and by stimulating angiogenesis to increase O2 delivery. These responses are principally mediated at the transcriptional level by hypoxia-inducible factor-1. This meeting emphasized the complexity of the tumor microenvironment and opportunities for therapeutic intervention by targeting transcriptional and metabolic pathways that are activated during cancer progression. A better understanding of the crosstalk between signaling pathways and metabolic alterations that contribute to the cancer phenotype may provide insights leading to the development of novel therapeutic strategies. (Cancer Res 2006; 66(9): 4558-60)

Introduction

One of the major conceptual advances in oncology over the last decade has been the appreciation that all major aspects of cancer biology are influenced by the tumor microenvironment. Features of the tumor microenvironment that are significantly different from normal tissue are the reduction in pO2 (hypoxia) and pH (acidosis) within tumors (1). Two important means by which cancer cells adapt to their microenvironment are by reprogramming cellular glucose/energy metabolism to use pathways that generate ATP in the absence of O2 and by stimulating angiogenesis to increase O2 delivery. These responses are principally mediated at the transcriptional level by hypoxia-inducible factor-1 (HIF-1; ref. 2). Recent progress in understanding the role of the tumor microenvironment in cancer progression was the subject of the 2nd International Tumor Metabolism Summit entitled "Exploiting the Tumor Microenvironment for Therapeutics," a meeting held at Palazzo Ducale in Genoa, Italy, October 7 to 8, 2005.

A.J. Giaccia (Stanford University) introduced the topic of hypoxia as a common feature of the tumor microenvironment and described the role of hypoxia-induced expression of the lysyl oxidase (LOX) gene in metastasis. LOX transcription is activated by the binding of HIF-1 to cis-acting hypoxia response elements in the LOX promoter. Genetic and pharmacologic manipulation of LOX expression and activity, respectively, indicated that LOX was a positive regulator of metastasis. This presentation and others discussed below provided new evidence for the role of HIF-1-regulated gene expression in promoting cancer cell migration, invasion, and metastasis. In addition to implicating LOX as a potential therapeutic target, Giaccia reported the preliminary results of an ongoing cell-based screen aimed at identifying compounds that selectively kill cancer cells that overexpress HIF-1.

Hypoxia and Biomarkers: the Role of Carbonic Anhydrase IX

In separate presentations, A.L. Harris (Oxford Cancer Center, United Kingdom) and E. Stanbridge (University of California, Irvine) discussed the role of carbonic anhydrase IX (CAIX), which is an important regulator of acid-base homeostasis and is encoded by a HIF-1-regulated gene (3). CAIX is coexpressed with HIF-1α in hypoxic areas, measured directly by oxygen electrodes, and is associated with poor prognosis. Knockdown of CAIX is also associated with poor survival of cancer cells cultured under hypoxic conditions. CAIX expression is also associated with poor transport of doxorubicin within the acidic microenvironment, reducing its uptake. Harris reported the results of a clinical analysis of neoadjuvant therapy with epirubicin at the maximum tolerated dose in breast cancer, which showed that patients with high CAIX had a poor outcome regardless of estrogen receptor status. This observation formed the basis of a new study using a combination of acetazolamide, celecoxib, and omeprazole to try to reduce hydrogen ion gradients and increase the effectiveness of epirubicin.

High levels of HIF-1α or HIF-2α expression are associated with poor prognosis in patients with head and neck cancer who received radiation therapy. Overall, patients with high HIF-1α and high HIF-2α expression had a particular poor outcome.

Hypoxia, Inflammation, and Macrophages

The role that HIF-1 may play in inflammation and immunity was elaborated by R.S. Johnson (University of California at San Diego), who presented recent findings from his laboratory, implicating HIF-1α in myeloid cell-mediated inflammation. HIF-1 is required for the production of several proinflammatory mediators, including nitric oxide and tumor necrosis factor-α. Mouse lacking HIF-1α in their myeloid lineage showed decreased bactericidal activity and failed to restrict systemic spread of infection from an initial tissue focus (4). HIF-1α expression is negatively regulated by the von-Hippel Lindau (VHL) tumor suppressor and deletion of VHL or pharmacologic induction of HIF-1α was associated with increased myeloid cell production of defense factors and bactericidal capacity. These results indicate that strategies to increase HIF-1α expression may...
enlarge innate immunity. With respect to the relationship between inflammation and cancer, C. Lewis (University of Sheffield, United Kingdom) emphasized the role of hypoxic macrophages in the secretion of growth/survival and angiogenic factors that may favor tumor progression. L. Varesio (G. Gaslini Institute, Genoa, Italy) discussed the transcriptional responses induced by exposure of mouse macrophages to hypoxia or to picolinic acid, a catalobate of tryptophan that chelates iron and thus induces HIF-1α expression. He reported that the gene encoding glutamine fructose-6-phosphate transaminase, the rate-limiting enzyme of the hexosamine pathway, is induced by either stimulus. These results have identified an additional metabolic pathway that may be modulated in an O2-dependent manner.

Role of Hypoxia and HIF-1 in Invasion and Migration

Accumulating evidence has implicated HIF-1 as a mediator of migration and invasion. G.L. Semenza (Johns Hopkins University School of Medicine, Baltimore, MD) reported that in VHL-null RCC cells, HIF-1-dependent loss of E-cadherin leads to decreased cell-cell adhesion, which is a prerequisite for tumor cell invasion and the defining characteristic of carcinomas. VHL loss of function resulted in a HIF-1-dependent increase in the expression of mRNAs encoding three transcriptional repressors of E-cadherin, thus providing a molecular mechanism for E-cadherin repression (5). M. Tran (Imperial College London, United Kingdom) presented similar results in RCC10 cells and presented immunohistochemical data indicating that loss of E-cadherin expression is an early event in the pathogenesis of renal cell carcinoma (RCC). Semenza also reported that both the chemokine receptor CXCR4 and its ligand stromal-derived factor 1 (SDF-1) are overexpressed in VHL-null RCC cells in a HIF-1-dependent manner. Intracellular expression of both proteins was detected in human RCCs and hemangio blasts in a renal biopsy from a patient with VHL disease, overexpression of CXCR4 and SDF-1 in the cytosol was associated with dramatic hypervascularization (6). Thus, CXCR4/SDF-1 autocrine or intracrine signaling may contribute to the angiogenic phenotype of these cancers. The involvement of the MET pathway in invasive growth was discussed by P.M. Comoglio (Institute for Cancer Research, Turin, Italy), who emphasized that invasive growth is a genetic program in which cell proliferation combines with cell-cell dissociation, migration, and apoptosis protection. MET is a proto-oncogene whose expression is regulated by unfavorable microenvironmental conditions, such as hypoxia. Activation of the MET receptor by the binding of hepatocyte growth factor, a protein closely related to blood coagulation factors, triggers cell motility and displacement toward more favorable tissue environments (7). MET activation induces the expression of genes involved in hemostasis, favoring tumor nesting in the newly colonized territories. This oncogene thus provides a functional mechanistic link among hypoxia, hemostasis, and invasive growth. Targeting the MET kinase receptor by recombinant “decoy” proteins, ligand antagonists, or antibodies may successfully block tumor onset and progression.

Hypoxia, Oncogenes, and Tumor Metabolism

C.V. Dang (Johns Hopkins University School of Medicine) reported that in addition to inducing expression of genes encoding glucose transporters and glycolytic enzymes, HIF-1 also induces the expression of PDK1, which encodes pyruvate dehydrogenase (PDH) kinase 1 (PDK1). PDK1 phosphorylates and inactivates PDH, the enzyme which catalyzes the conversion of pyruvate to acetyl CoA. Incubation of mouse embryo fibroblasts (MEF) that lack HIF-1α expression under prolonged hypoxic conditions leads to excessive production of reactive oxygen species (ROS) and apoptotic cell death. Transfection of HIF-1α null MEF with a PDK1 expression vector reduces ROS generation and prevents apoptosis under hypoxic conditions. Thus, HIF-1 modulates four critical steps in glucose metabolism by increasing glucose transport into cells, increasing the conversion of glucose to pyruvate, increasing the conversion of pyruvate to lactate, and decreasing the conversion of pyruvate to acetyl CoA.

J. Coy (R-Biopharm AG, Darmstadt, Germany) discussed the role of the pentose phosphate pathway in the metabolism of glucose by cancer cells. The TKT, TKTL1, and TKTL2 genes encode transketolase and transketolase-like proteins that may participate in the pentose phosphate pathway. A mutated TKTL1 mRNA was detected in human cancer cells and invasive bladder and colon cancers expressed high levels of TKTL1 protein. These intriguing results suggest that the role of the pentose phosphate pathway in cancer requires further investigation. C. Godinot (Centre National de la Recherche Scientifique-Universite Claude Bernard de Lyon 1, Villeurbanne, France) reported reduced expression and activity of electron transport chain components and decreased mitochondrial DNA content in VHL-null renal carcinoma cells compared with subclones transfected with a VHL expression vector (8). Expression of mitochondrial transcription factor A was also reduced in VHL-null compared with VHL-rescued cells and may contribute to the loss of mitochondrial DNA and mitochondrially encoded gene products.

Different conditions of the tumor microenvironment may affect the growth of cancer cells, and Jacques Pouyssegur (CRNS Nice, France) discussed how pH of tumor tissue might not only affect the survival of cancer cells but may also be exploited therapeutically. It is well recognized that tumor cells have high levels of aerobic glycolysis, also known as Warburg effect, a phenomenon which is at least in part determined by overexpression of HIF-1. However, Pouyssegur emphasized that a defect in glycolysis of Ras-transformed fibroblasts did not alter tumor growth, whereas respiration defective mutants, which rely on glycolysis for energy supply, were significantly impaired. In addition, mutation of the Na-H exchanger (NHE1), a central pH-regulating system, was associated with impaired tumor growth, a finding consistent with the conclusion that tumor suppression may be achieved by controlling pH regulation and exploiting the acidosis of highly glycolytic tumors. Specific inhibitors for NHE1, HCO3-/Cl- exchangers, lactate/H+ symporter, and/or blockers of carbonic anhydrases may therefore be useful anticancer agents. Pouyssegur anticipated that combining pH-targeted therapy with antiangiogenic therapy to increase hypoxia-mediated glycolysis might be particularly effective.

Targeting the Tumor Microenvironment

G. Melillo (National Cancer Institute, Frederick, MD) described high-throughput screening assays designed to identify small-molecule inhibitors of HIF-1. Using a cell-based assay involving U251 human glioma cells that stably express a recombinant vector in which the luciferase reporter gene is under control of hypoxia-responsive elements, toposomerase I inhibitors, such as topotecan, were found to potently inhibit expression of HIF-1α protein (9). Topotecan inhibits HIF-1α protein translation through an unknown mechanism that is dependent on its activity as a
topoisomerase I inhibitor. Daily administration of topotecan inhibited HIF-1α expression, angiogenesis, and growth of glioma xenografts (10), thus providing a rationale for an ongoing clinical trial of topotecan in patients with cancers that have been shown to overexpress HIF-1α by immunohistochemistry. A second screening assay for compounds that inhibit HIF-1 DNA binding activity in vitro led to the identification of echinomycin as a novel inhibitor of HIF-1-dependent transcription (11). Echinomycin binds to the DNA sequence 5′-ACGT-3′, which is present in a subset of binding sites recognized by HIF-1 (5′-ACGTG-3′) and C-MYC (5′-ACAGTG-3′).

D. Del Bufalo (Regina Elena Cancer Institute, Rome, Italy) extended recent observations by her lab that Bcl2 overexpression increases vascular endothelial growth factor (VEGF) via HIF-1-dependent transcription by reporting that Bcl2 overexpression stimulated the phosphorylation of AKT and extracellular signal-regulated kinase 1/2 (ERK1/2) proteins under hypoxic conditions, whereas Bcl2 had no effect on these proteins under nonhypoxic conditions. Bcl2 RNA interference decreased ERK1/2 phosphorylation and VEGF secretion in hypoxic Bcl2-overexpressing cells but not in control cells (12). Thus, Bcl2 overexpression and hypoxia have synergistic effects on HIF-1-dependent gene expression in tumor cells. Finally, P. Burke (EntreMed, Inc., Rockville, MD) reported that

2-methoxyestradiol and a derivative (ENMD-1198) destabilize microtubules, inhibit HIF-1α accumulation, and block tumor proliferation and angiogenesis in an MDA-MB-231 human breast cancer xenograft model. Remarkably, in addition to inhibiting HIF-1, these drugs were shown to block the activation of signal transducers and activators of transcription 3 and nuclear factor-κB, two other transcription factors whose dysregulation is believed to play an important role in the pathogenesis of many cancers.

In conclusion, this meeting emphasized the complexity of the tumor microenvironment and opportunities for therapeutic intervention by targeting transcriptional and metabolic pathways that are activated during cancer progression. A better understanding of the crosstalk between signaling pathways and metabolic alterations that contribute to the cancer phenotype may provide insights leading to the development of novel therapeutic strategies.

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References

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