HIGD1A-mediated dormancy and tumor survival

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Solid tumors contain regions of anoxia that are also glucose deprived. How cancer cells survive such extreme conditions remains unclear. Here, we discuss our recent findings that regulation of hypoxia inducible gene domain family member 1 A (HIGD1A) via epigenetic mechanisms during glucose starvation modulates oxygen consumption and reactive oxygen species production to enable tumor cell survival through the activation of dormancy mechanisms.

Introduction

Tumors are highly heterogeneous with respect to both genetics and metabolism.^{1,2} In solid tumors, some regions exhibit extremely low, or near anoxic, levels of oxygen. These regions are also typically nutrient starved as a result of the irregular vascularity frequently observed in solid tumors.³ The cores of solid tumors are therefore frequently necrotic and surrounded by a rim of viable but highly stressed tissue known as the perinecrotic region. Paradoxically, these regions frequently lack expression of hypoxia-inducible factor-1 α (HIF-1 α), an important transcriptional regulator of cellular responses to oxygen deprivation.⁴ Lack of HIF-1 α in these severely hypoxic regions may be due to simultaneous glucose deprivation.⁵ These observations suggest that additional survival mechanisms must be operative in oxygen/nutrient starved tumor regions that lack HIF-1 α .

Hypoxia inducible gene domain family member 1 A (HIGD1A) is a HIF-1 α targeted mitochondrial protein that is typically induced by hypoxia.⁶ We recently showed that human cancer cells do not induce expression of *HIGD1A* during

hypoxia despite the presence of hypoxia response elements within its promoter that are able to bind HIF-1.⁷ Interestingly, however, we noted enhanced expression of HIGD1A in perinecrotic tumor regions in tumor xenograft models, indicating HIF-independent induction. Therefore, enhanced HIGD1A expression was noted in conditions where cells may survive by triggering dormancy mechanisms in a HIF-independent manner. To model this state, we engineered HIF-1-deficient transformed mouse embryonic fibroblasts to constitutively express HIGD1A. Indeed, when these cells were grown as xenografts, they developed dramatically smaller tumors than their control HIF-1-deficient counterparts and remained as barely palpable masses following 3 weeks of growth. Importantly, these smaller tumors exhibited diminished apoptosis and did not show signs of necrosis, implying enhanced tumor cell survival, possibly by inducing a state of metabolic dormancy.⁷ Consistent with this assertion, *in vitro* analyses indicated that the same HIGD1A overexpressing cells exhibited lower oxygen consumption and cellular levels of reactive oxygen species (ROS) during glucose starvation. We demonstrated that this is likely due to the ability of HIGD1A to directly interact with Complex III of the mitochondrial electron transport chain, which is critical for both respiration and ROS formation. Thus, HIGD1A may promote tumor dormancy by repressing tumor metabolism under conditions of severe metabolic stress (Fig. 1).

However, this response would not be desirable under less severe tumor environments that do not limit growth. In this setting, HIGD1A induction would have tumor suppressor effects, whereby

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Abbreviations: DNMT1, DNA methyltransferase 1; HIGD1A, hypoxia inducible gene domain family member 1 A; HIF-1, hypoxia-inducible factor-1

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Figure 1. Regulation of tumor dormancy in response to metabolic stress by HIGD1A.

its actions would restrain tumor growth under conditions of mild to moderate tumor hypoxia that are otherwise permissive for growth. It might be predicted, therefore, that enhanced expression of HIGD1A would be prevented under conditions of physiological hypoxia that induce other HIF-1 target genes. Conversely, in conditions of more severe metabolic compromise HIGD1A might act as an oncogene, preserving tumor cell viability by activating dormancy mechanisms. Thus, tumor evolution may have selected mechanisms that prevent HIF-1-mediated expression of HIGD1A to enable growth during mild to moderate hypoxia, but allow its expression in more severe metabolic stress conditions to allow dormancy-mediated survival.

Induction of long-term tumor repression or quiescence can involve epigenetic mechanisms.⁸ Consistent with this, our analysis of gene regulatory regions indicated that the human *HIGD1A* gene promoter harbors a differentially methylated region in many transformed human cancer cell lines. HIF-1 can bind this region during moderate hypoxia, but fails to activate its expression. However, other

HIF-1 target genes were induced by moderate hypoxia in these cells. We found that pharmacological demethylating agents or glucose starvation-mediated downregulation of DNA methyl-transferase (DNMT) expression triggered HIGD1A expression in these cells in a HIF-1-independent manner. Thus, perinecrotic tumor regions presumably also induce HIGD1A expression via similar mechanisms. Whether other genes that are also induced in these settings, such as carbonic anhydrase IX,⁴ utilize this mechanism is not known. In leukemic and epithelial cancer cells, the DNA methylation inhibitor 5-azacytidine was shown to decrease expression of G0 to G1 exit genes, including Forkhead box protein M1 (*FOXM1*) or *DNMT1*, which are downregulated in tumor dormancy models.⁸ Thus, DNA demethylation may either induce (e.g., *HIGD1A*) or repress (e.g., *FOXM1*, *DNMT1*) genes in a manner that promotes tumor cell quiescence and dormancy. These processes likely take place *in vivo* after various therapeutic strategies that are designed to inhibit tumor growth but simultaneously trigger widespread ischemia, such as antiangiogenesis treatment.³

Indeed, we found a dramatic increase in HIGD1A expression following bevacizumab therapy in human glioblastomas *in vivo*.⁷

One of the least understood aspects of cancer biology is the remarkable ability of cancers to recur years following therapeutic intervention. After removal of the primary tumor and/or chemotherapy and irradiation, small numbers of residual tumor cells are presumably maintained in a state of dormancy and thereby evade therapies targeting rapidly proliferating cancer cells.⁹ Sometimes these cells can enter the circulation and migrate to distant sites, contributing to tumor metastasis. Our results therefore have potential implications for cancer biology. Approaches targeting HIF activity to impair tumor growth may inadvertently trigger tumor dormancy mechanisms that could ultimately result in tumor recurrence with more aggressive phenotypes. Approaches aimed at modulating HIGD1A activity in anoxic tumor regions may help overcome dormancy-mediated survival observed in the setting of antiangiogenesis or potentially following HIF inhibition. Small molecule modulators of the HIGD family of mitochondrial proteins are being investigated¹⁰ and may hold promise in this capacity.

Solid tumors frequently exhibit necrotic cores as a result of very low oxygen and nutrient levels. Surrounding these cores, a rim of perinecrotic tissue remains viable but is exposed to quite severe oxygen/nutrient deprivation. In these settings, HIF-1 is repressed, likely due to glucose deprivation, and HIGD1A is induced, likely due to DNMT1 repression and decreased methylation of the *HIGD1A* gene promoter. HIGD1A promotes survival and dormancy by repressing oxygen consumption. More distal regions are exposed to higher oxygen and nutrient levels. These environments enable HIF-1 induction but repress HIGD1A by activating DNMT1 expression to enable rapid growth.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

- McGranahan N, Swanton C. Biological and therapeutic impact of intratumor heterogeneity in cancer evolution. *Cancer Cell* 2015; 27, 15–26; PMID:25584892; <http://dx.doi.org/10.1016/j.ccr.2014.12.001>
- Mayers JR, Vander Heiden MG. Famine versus feast: understanding the metabolism of tumors in vivo. *Trends Biochem Sci* 2015; 40, 130–140; PMID:25639751; <http://dx.doi.org/10.1016/j.tibs.2015.01.004>
- McIntyre A, Harris AL. Metabolic and hypoxic adaptation to anti-angiogenic therapy: a target for induced essentiality. *EMBO Mol Med*. 2015 Feb 19; 7(4):368–379
- Sobhanifar S, Aquino-Parsons C, Stanbridge EJ, Olive P. Reduced expression of hypoxia-inducible factor-1alpha in perinecrotic regions of solid tumors. *Cancer Res* 2005; 65, 7259–7266; PMID:16103077; <http://dx.doi.org/10.1158/0008-5472.CAN-04-4480>
- Osada-Oka M, Hashiba Y, Akiba S, Imaoka S, Sato T. Glucose is necessary for stabilization of hypoxia-inducible factor-1alpha under hypoxia: contribution of the pentose phosphate pathway to this stabilization. *FEBS Lett* 2010; 584, 3073–3079; PMID:20621833; <http://dx.doi.org/10.1016/j.febslet.2010.05.046>
- Ameri K, Rajah AM, Nguyen V, Sanders TA, Jahangiri A, Delay M, Donne M, Choi HJ, Tormos KV, Yeghiazarians Y, Jeffrey SS, et al. Nuclear localization of the mitochondrial factor HIGD1A during metabolic stress. *PLoS One* 2013; 8, e62758; PMID:23646141; <http://dx.doi.org/10.1371/journal.pone.0062758>
- Ameri K, Jahangiri A, Rajah AM, Tormos KV, Nagarajan R, Pekmezci M, Nguyen V, Wheeler ML, Murphy MP, Sanders TA, et al. HIGD1A Regulates Oxygen Consumption, ROS Production, and AMPK Activity during Glucose Deprivation to Modulate Cell Survival and Tumor Growth. *Cell Rep* 2015 Vol. 10 Issue 6, 891–899; PMID:25683712
- Tsai HC, Li H, Van Neste L, Cai Y, Robert C, Rassool FV, Shin JJ, Harbom KM, Beaty R, Pappou E, et al. Transient low doses of DNA-demethylating agents exert durable antitumor effects on hematological and epithelial tumor cells. *Cancer Cell* 2012; 21, 430–446; PMID:22439938; <http://dx.doi.org/10.1016/j.ccr.2011.12.029>
- Sosa MS, Bragado P, Debnath J, Aguirre-Ghiso JA. Regulation of tumor cell dormancy by tissue microenvironments and autophagy. *Adv Exp Med Biol* 2013; 734, 73–89; PMID:23143976; http://dx.doi.org/10.1007/978-1-4614-1445-2_5
- Lindert S, Maslennikov I, Chiu EJ, Pierce LC, McCammon JA, Choe S, et al. Drug screening strategy for human membrane proteins: from NMR protein backbone structure to in silica- and NMR-screened hits. *Biochem Biophys Res Commun* 2014; 445, 724–733; PMID:24525125; <http://dx.doi.org/10.1016/j.bbrc.2014.01.179>