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Hypoxic regulation of metabolism offers new opportunities for anticancer therapy

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Abstract

Cancer metabolism now appears to be optimized for growth of tumor cells by having an increased reliance on non-oxidative processes. However, in order to exploit these findings clinically, we must determine the specific pathways and components that cancer cells rely on, but are dispensable for normal cells. Because tumors have the added stress of hypoxia, the metabolic response to low oxygen may represent such a tumor-specific metabolic program.

Keywords

α-ketoglutarate dehydrogenase; pyruvate dehydrogenase kinase

There has been a resurgence of interest in the study of tumor metabolism in recent years [1–3]. These studies have yielded significant insight into the causes and consequences of the reliance of tumor cells on glycolysis at the expense of mitochondrial oxidation. In addition to the driving genetic changes in the genome that direct metabolism, it is now becoming clear that environmental conditions contribute to metabolic changes as well. Environmental conditions can limit delivery of metabolic substrates such as glucose or oxygen to the tumor cell. In addition, stromal cells can metabolically process environmental substrates, for example, conversion of glucose into lactate which may or may not be utilized by the tumor cells. Hypoxia has become recognized as an environmental condition found in the solid tumor that modifies cellular metabolism to amplify the Warburg characteristics. These changes not only impact the flux of glucose to pyruvate, but also determine how glucose can be used biosynthetically in other processes. For example, increased glucose flux into the pentose phosphate pathway increases the synthesis of purines needed for nucleotide synthesis, and the NADPH reducing equivalents that are essential for *de novo* fatty acid synthesis and buffering of redox stress [3]. Hypoxia can cause both increased glycolysis and decreased mitochondrial oxidative phosphorylation. The hypoxia inducible factor-1(HIF-1) transcription factor appears to be responsible for both these metabolic changes, through the

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induction of numerous target genes. These pathways shift energy production and macromolecular synthesis of the growing tumor cells to match the available substrates found in the hypoxic tumor microenvironment.

It now appears that it is not just the elevated glucose uptake and glycolysis that enhances tumor cell growth *in vivo*, but also the ability to decrease mitochondrial oxidation that is essential to the growth of model tumors. Several groups have tried a general strategy to block tumor cell glucose consumption with limited therapeutic benefits. However, recent investigation of hypoxia-dependent reduction in oxidative phosphorylation has shown this change is also very important for tumor growth. Delineating the upstream signals that lead to adaptation of mitochondrial function to environmental hypoxia may offer new opportunities for therapeutic intervention.

Tumor cells adapt to hypoxic stress *in vivo*

As tumors outgrow their blood supply, the limited amount of oxygen delivered to the tumor is not sufficient for the cellular demand. This supply–demand imbalance results in a hypoxic microenvironment in the regions of the tumor that are at a significant distance away from the feeding blood vessel. The cancer cells must adapt to the lack of oxygen by altering their metabolism so that they can still produce macromolecules for growth and not consume all the oxygen and induce death by anoxia. Most tumor cells will proliferate quite well in intermediate hypoxia, as long as sufficient metabolites are available in the media. However, the environmental milieu in regions of the tumor that are hypoperfused is not only hypoxic but also suffers from reduced metabolites and increased waste products (hypoglycemia and acidosis), further adding to the cellular stress.

One of the major mechanisms by which cells adapt to hypoxia is through the induction of the HIF family of transcriptional activators. HIF-1 is a heterodimeric transcription factor made up of a hypoxia-inducible α subunit and a constitutive α subunit. It was recognized early on that HIF-1 stabilization in hypoxia could induce expression of many of the genes of the glycolytic family of enzymes [4]. The induction of glycolysis in hypoxia has since been studied extensively by a number of groups. More recently, it has become apparent that hypoxia also leads to the downregulation of mitochondrial oxidation as well, further enhancing the Warburg-like reliance of tumor cells on glycolytic energy production [5]. The reduction in oxidative phosphorylation also leads to a reduction in the generation of endogenous reactive oxygen species (ROS) which can be formed as a byproduct of electron transport. Decreasing ROS generation reduces oxidative stress in the hypoxic cell, but also has pleitrophic effects as ROS can act as an intracellular signal and an oxidizer of enzymes within the cell [6]. Most interesting is the fact that it is the mitochondrial adaptation to hypoxia that is necessary for model tumor growth [7–9]. One does not need to entirely block the metabolism of either glucose or glutamine or destroy all the mitochondrial functions to stop the growth of model tumors; rather simply turn ‘on’ the mitochondria when hypoxia has tried to turn them ‘off’.

Mechanisms of hypoxic reduction of oxidative phosphorylation

It was recognized several years ago that HIF-1 stabilization induces the transcription of the pyruvate dehydrogenase kinase genes 1 and 3 [10–12]. These kinases phosphorylate and inactivate the E1α subunit of pyruvate dehydrogenase, and block pyruvate entry into the tricarboxylic acid (TCA) cycle. This has the effect of reducing mitochondrial oxygen consumption and increasing cellular level of pyruvate, which is used to generate lactate and NAD+. Lactate is released into the extracelluar space, and NAD+ is used as a substrate for more glycolysis. The regulation of PDH by hypoxia is an effective way for cells in a hypoxic microenvironment to downregulate the consumption of oxygen, thereby conserving a scarce resource. However, reduction in PDHK activity and increase in pyruvate entry into the TCA cycle have been reported to block the growth of model tumors without affecting cellular growth *in vitro* [8]. The precise mechanism for this block to tumor growth has not yet been identified.

However, for proliferating cells, there is a need for intermediates of the TCA cycle for biosynthetic purposes. Most important for proliferating cells is citrate, which is used in the cytoplasm as a precursor for the synthesis of fatty acids and lipids which are necessary for the production of membranes. Recent work has elegantly shown how proliferating cells in hypoxia can tolerate the reduction in glucose-derived citrate by substituting glutamine as a second carbon source for anaplerosis [13]. Through the process of reductive carboxylation, gluta-mine is converted into isocitrate and citrate to be used in the *de novo* generation of fatty acids. It has only been very recently identified by us how the reductive carboxylation of glutamine is regulated in hypoxia and how important it is to the growth of model tumors [9]. In hypoxia, HIF-1 activates the E3 ubiquitin ligase SIAH2, leading to proteolytic degradation of the OGDH2 component of the a-ketoglutarate dehydrogenase complex in the TCA cycle. Without the ‘forward’ TCA reaction, α-ketoglutarate builds up and by mass action, reverses the isocitrate dehydrogenase reaction. The ‘reverse’ reaction produces citrate necessary for fatty acid production. Expression of an OGDH2 protein in which the ubiquitinated lysine has been mutated blocks the hypoxic reductive carboxylation of aketoglutarate and maintains glutamine oxidation. These cells are unable to proliferate in hypoxia without the addition of cit-rate or fatty acids to the media. Interestingly, they also do not grow as tumors in immune-deficient mice, demonstrating that *de novo* production of fatty acids is essential in hypoxic tumor cells *in vivo*.

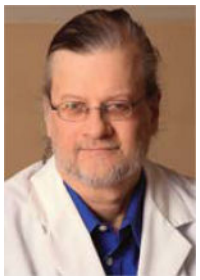
PDHK1 and OGDH2, therefore, represent two different mechanisms by which hypoxia downregulates mitochondrial oxidative phosphorylation by reducing the substrates available to the mitochondria. It has now been shown genetically that both these processes are necessary for the growth of model tumors, but not for the *in vitro* growth of cells in normoxia. These processes represent attractive targets for anticancer drug development because they should only be active in the poorly oxygenated tumors. Pharmacological inhibition of these hypoxic pathways should not cause the unwanted side effects that are common after the inhibition of a more widely active pathway like glycolysis. The brain and muscle both rely upon glycolysis for normal function, so trials of general glycolytic inhibitors, such as 2-deoxyglucose, have had to be stopped due to side effects. Likewise, glutamine participates in neurotransmission, so blocking all glutamine metabolic pathways

would also risk significant side effects. However, increasing the oxidation of either of these metabolites would only serve to stimulate a process that is ongoing in normal tissue.

Recent reports examining the use of dichloroacetate (DCA) have suggested that increasing mitochondrial function as an anticancer approach may have merit. DCA is an inhibitor of the PDHKs at millimolar concentrations. In some animal studies, DCA has shown anticancer activity; however, the results are mixed [14]. However, it does not seem possible to achieve high enough doses of DCA in humans to inhibit the PDHKs. Certainly a more potent DCA, or one that specifically inhibited PDHK1, would have the potential as a novel metabolic anti-cancer agent. It is important to recognize that PDHKs are important in hematopoietic stem cell renewal, and bone marrow may represent a site for the dose-limiting toxicity of such a molecule [15]. Because glutamine metabolism is regulated by proteolysis, it appears to be a more difficult pathway ‘to drug’. Perhaps better understanding of upstream signals may offer a site for drug development. It appears that as our understanding of metabolic regulation advances, it may be more fruitful to just redirect metabolism for anticancer efficacy, rather than blocking it entirely.

Biography

Nicholas C Denko



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