Hypoxia and Enzyme Metabolism: A Review

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Abstract: Hypoxia increases the production of hypoxia–inducible factor (HIF-1) which acts a major regulatory transcription factor responsible for some cellular changes via its effects on glycolytic genes to cope with the reduction in oxygen availability and consumption. This affects the enzymes involved in metabolism which affects the products of metabolism. This review throws light on the changes on enzyme metabolism induced by hypoxia.

Key words: Hypoxia · Enzyme · Metabolism · Metabolic Pathways

INTRODUCTION

Metabolic change, historically known as the Warburg effect [1] results in high rates of glycolysis in both normoxic and hypoxic cancer cells. Expression of genes responsible for glycolytic enzymes and glucose transporters are enhanced by numerous oncogenes including RAS, SRC and MYC [2].

Traditionally, hypoxia leads to increased production of hypoxia-inducible factor (HIF-1), containing HIF-1α and HIF-1β subunits that acts as a key regulatory transcription factor responsible for adaptive cellular changes. In humans, HIF-1 has been shown to up-regulate expression of genes affecting a range of target areas of physiology. These genes range from those involved in triggering an inflammatory response to those responsible for iron metabolism. Particularly notable when focusing on metabolism, HIF-1 is shown to affect glycolytic genes to cope with reductions in oxygen availability and consumption.

These genes include: solute carrier family 2 (GLUT1), hexokinase (HK), phosphoglucose isomerase (PGI), phosphofructokinase (PFKL), fructose-bisphosphate aldolase (ALDO), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), phosphoglycerate mutase (PGM), enolase 1 (ENOA), pyruvate kinase (PK), pyruvate dehydrogenase kinase, isozyme 1 (PDK1) and lactate dehydrogenase A (LDH-A).

In addition to alterations in oxygen concentration associated with hypoxic microenvironments, glucose concentration gradients found in tumors also influence the rate of aerobic and anaerobic glycolysis. A carbohydrate-response element (ChoRE) is responsible for regulating glycolytic enzyme gene expression in response to changing glucose concentrations through a binding interaction at the same consensus sequence as HIF-1. Interactions of HIF-1 and ChoRE with the DNA sequence 5'-RCGTG-3' leads to increased expression of genes listed above [3].

GLUT1 is a member of the GLUT transporter family of 14 hexose transporters responsible for facilitating the transport of hexose sugars along the concentration gradient. GLUT1 is the most abundantly expressed of the family thought to maintain basal glucose transport in almost all cell types. GLUT1 levels, in response to hypoxic conditions, have been shown to increase with changes at both the mRNA and protein levels. Moreover, transport of GLUT1 has been shown to increase under these hypoxic conditions. With the role of transporting sugars from the extracellular to the intracellular environment, GLUT1, along with other members of the GLUT family, can be rate-controlling for cellular glycolytic metabolism. Having an increased level of GLUT1, in the case of hypoxic tumors, increases the flux of glucose into the cells allowing for a higher rate of glycolysis and thus greater risks of metastasis [4].

Hexokinase 2 Expression: Hexokinase (HK) is the first enzyme in the glycolytic pathway converting glucose to glucose-6-phosphate through an ATP-dependent phosphorylation event. Important for glycolysis to

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proceed, the hexokinase reaction activates glucose for subsequent steps. In hypoxic tumors, hexokinase mRNA abundance is significantly increased as well as protein levels. Increased expression of hexokinase 2, in some cases nearly 10-fold, allows for an increased flux of glucose through the glycolytic pathway subsequent to the increased uptake by GLUT1 [5].

Phosphoglucose Isomerase Expression: Phosphoglucose isomerase (PGI) is a housekeeping cytosolic enzyme with roles in both glycolysis and gluconeogenesis pathways. It is responsible for catalyzing the interconversion of glucose 6-phosphate and fructose 6-phosphate. Extracellularly, PGI is known as an autocrine motility factor (AMF) eliciting mitogenic, motogenic, differentiation functions as well as tumor progression and metastasis [6]. Activation of PGI through proposed HIF-1 induced mechanisms results in increased conversion of glucose 6-phosphate to fructose 6-phosphate and also contributes to cell motility and invasion during cancer metastasis.

6-Phosphofructo-2-kinase/fructose 2,6-Bisphosphatases Expression: 6-Phosphofructo-2-kinases/fructose 2,6-bisphosphatases (PFKFBs) belong to a family of bifunctional ATP-dependent enzymes responsible for controlling the level of glycolysis intermediate fructose-1,6-bisphosphate. HIF-1-induced expression of these enzymes (PFK-2/FBPase-2) subsequently alters the balance of fructose-2,6-bisphosphate which plays an important role as an allosteric activator of phospho-fructokinase 1 (PFK-1). PFK-1 is an enzyme that controls one of the most critical steps of glycolysis. Regulation of PFK-1 is also mediated by the cellular energy status in result of ATP's inhibitory effect. Greater quantities of fructose-2,6-bisphosphate in cancer cells, in result of HIF-1 expression of PFK-2/FBPase-2, thus activates PFK-1allowing for an increased glycolytic flux converting fructose-6-phosphate to fructose-1,6-bisphosphate. Allosteric regulation of glycolysis by fructose-2, 6-bisphosphate allows cancer cells to maintain a glycolytic balance to match their bioenergetic and biosynthetic demands [7].

Fructose-1, 6-Bisphosphate Aldolase Expression: Fructose-1,6-bisphosphate aldolase (ALDO) belongs to a family include aldolase A, B and C. Unique in glycolysis, aldolase enzymes cleave fructose-1,6-bisphosphate into two 3-C molecules including glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP). With the HIF-1 mediated expression of aldolase A under

hypoxic conditions, the catalysis of fructose-2,6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate is increased thus leading to increased glycolytic flux.

Glyceraldehyde-3-Phosphate Dehydrogenase Expression: The glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), is responsible for the oxidative conversion of glyceraldehyde-3-phosphate (GADP) to 1,3-bisphosphoglycerate (1,3BPG). Up-regulation of glyceraldehyde-3-phosphate dehydrogenase expression is maximal (4-5 fold) following hypoxic conditions of ~24 hours in vascular endothelial cells [8]. Various models for the exact glyceraldehyde-3-phosphate dehydrogenase activation mechanisms have been proposed.

Phosphoglycerate Kinase 1 Expression: Hypoxia has been shown to induce a 10-fold accumulation of phosphoglycerate kinase 1 (PGK-1) mRNA in mouse hepatoma (Hepa 1c1c7) cells. Phosphoglycerate kinase 1 is an enzyme involved in the conversion of 1, 3-bisphosphoglycerate (1,3-BPG) to 3-phosphoglycerate (3-P-G) leading the production of ATP from ADP. Induction of gene expression by HIF-1 is thought to be dependent on the presence of aromatic hydrocarbon receptor nuclear translocator (ARNT1). Arnt's N-terminal region and HIF-1 are thought to work together to induce transcription of phosphoglycerate kinase 1 [9].

Phosphoglycerate Mutase Expression: Phosphoglycerate mutase B (PGM-B) is one of the latter glycolytic enzymes responsible for the conversion of 3-phosphoglycerate (3PG) to 2-phosphoglycerate (2PG). Both protein and mRNA levels were shown to increase 2-3-fold in research exposing fetal rat lung fibroblasts to hypoxic conditions. Increased levels appeared to be regulated at the transcriptional level as per many of the other glycolytic enzymes. Maximum up regulation was shown following 16 hours thus supporting its role in contributing to an increased glycolytic flux for adaption of cells to hypoxia [10].

Enolase 1 Expression: Enolase 1, also known as α-enolase, is encoded by the ENOA gene and is responsible for converting 2-phosphoglycerate to phosphoenolpyruvate in the glycolytic pathway. Both enolase 1 overexpression and its post-translational modifications could be of value for diagnostic and prognostic work in terms of cancer. Although the exact roles of post-translational modifications have not been completely elucidated, patterns are shown between

certain cancer cell types suggesting they may have important influence on function, localization and immunogenicity. Aside from its role in promoting glycolytic flux and anearobic energy production, it has been shown to induce a specific humoral and cellular immune response. On all levels, hypoxia-induced over-expression of enolase 1 may possess important roles in hypoxic tumors including the most straightforward increase in anearobic respiration.

Pyruvate Kinase Expression: HIF-1 activated pyruvate kinase M comes in multiple isoforms known as PKM1 and PKM2. Pyruvate kinase is shown to convert phosphoenolpyruvate to pyruvate forming ATP from ADP. Along with phospho-fructokinase 1, pyruvate kinase is also allosterically activated by fructose-2, 6-bisphosphate. In cancer cells pyruvate kinase M2 has been shown to interact directly with HIF-1α enhancing HIF-1 binding and p300 recruitment to hypoxia response elements. This positive feedback loop leads to HIF-1 transactivation and an amplified effect on glucose metabolism [11].

Pyruvate kinase M2 is often considered the main regulator of cancer metabolism with roles in various parallel, feed-forward and positive and negative feedback mechanisms. The genetic difference between pyruvate kinase M1 and pyruvate kinase M2 is only 22 out of 531 amino acids which makes an immense difference. Pyruvate kinase M2 has metabolic activity regulated by post-translational modifications including acetylation, oxidation, phosphorylation, hydroxylation and sumoylation. These different modifications can cause the shift from the metabolically active tetrameric form to the in-active monomeric form. The well-known EGFR-activated extracellular signal-regulated kinase 2 (ERK2) and death-associated protein kinase are both shown to bind and directly phosphorylate pyruvate kinase M2 leading to increased activity in the glycolysis pathway [12]. In hypoxic conditions found in a solid tumor, pyruvate kinase M2 plays a large role in promoting anearobic energy production.

The areas surrounding the phosphorylation sites on pyruvate dehydrogenase are shown in red. Pyruvate dehydrogenase kinase phosphorylation of these sites leads to decreased dehydrogenase activity

Pyruvate Dehydrogenase Kinase Expression: Pyruvate dehydrogenase directly follows the glycolytic pathway and is responsible for the conversion of pyruvate to acetyl-CoA which enters into the TCA cycle. The TCA cycle, although not directly requiring oxygen, requires the cycling of NADH to NAD+ as performed by the electron transport chain under aerobic conditions. Under anaerobic conditions, such as those found in hypoxic tumors, the TCA cycle provides little ATP yield due to the lack of electron transport chain function. In order to direct the glycolytically produced pyruvate away from the TCA cycle, pyruvate dehydrogenase kinase is over-expressed in response to hypoxic conditions. Pyruvate dehydrogenase kinase is not a glycolytic enzymes but more of a glycolytic regulator. Pyruvate dehydrogenase kinases, transcriptionally activated by HIF-1 in hypoxic conditions, are responsible for phosphorylating the E1 subunit of pyruvate dehydrogenase ultimately suppressing its function. By inhibiting this specific pathway, the glycolytic products are directed away from the mitochondrial TCA cycle and towards lactate dehydrogenase [13].

Lactate Dehydrogenase Expression: Activated expression of lactate dehydrogenase A (LDH-A), parallels with deactivation of pyruvate dehydrogenase mediated by pyruvate dehydrogenase kinase. Subsequent inactivation of pyruvate dehydrogenase following phosphorylation and increased expression of lactate dehydrogenase A shunts pyruvate away from the mitochondrial TCA cycle.

The Pentose Phosphate Pathway: They must coordinate production of precursors for macromolecular synthesis as well as maintain cellular bioenergetics without impairing cell growth, proliferation and viability. One way of doing this is by shuffling glycolytic intermediates such as glucose-6-phosphate into the pentose phosphate pathway to give ribose-5-phosphate and NADPH. Ribose-5-phosphate acts as an intermediate for the production of nucleotides thus providing a connection between glycolysis and nucleotide synthesis in hypoxic cells. In cases where glycolysis remains highly active in normoxic conditions, NADPH acts as a mediator of antioxidative reactions to protect cells from oxidative damage.

CONCLUSION

Warburg effect leads to high rates of glycolysis in both nrmoxic and hypoxic cancer cells. Hypoxia increases the production of HIF-1 which regulates the changes associated with hypoxia which in turn affects up-regulation expression of genes affecting a range of targets areas of physiology. This results in serious changes in the enzymes in the meatabolic pathways. Therefore, everything should be done to avoid

hypoxic condition to avert its effect on metabolism because the cells depends on these vital metabolism for the generation of ATP.

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