Genetic selection for enhanced serine metabolism in cancer development

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Most tumors display enhanced rates of glucose metabolism compared with that of normal differentiated tissues. The preferential conversion of glucose to lactate in cancer cells (the Warburg effect) was one of the first known differences between tumor and normal cells and is believed to contribute to enhanced growth in tumor cells.[1-3] However, the extent to which specific metabolic fluxes that branch from central carbon metabolism are involved in tumor growth is poorly understood. It has also recently been proposed that metabolic enzymes can function as oncogenes and tumor suppressor genes,[4] but, it is also not well-understood as to whether an enhanced flux of glucose into peripheral metabolism contributes to the development of human cancer. In a recent study,[5] we used an integrative quantitative metabolomics approach, combining NMR experiments with heavy isotope labeling and targeted mass spectrometry to investigate this question. We performed direct measurements of metabolic fluxes emanating from glucose metabolism.

It was found that in some cancer cells, a relatively large amount of glycolytic carbon was diverted into serine and glycine biosynthesis (**Fig. 1**). Serine can be synthesized from a glycolytic intermediate, 3-phosphoglycerate (3PG).[6] A genetic context in which PHGDH protein expression and altered flux through this pathway might be driving cancer development emerged from a pooled analysis of 3131 human cancers.[7] This analysis revealed that *PHGDH*, the gene that encodes the first enzyme in this biosynthetic pathway branching off of glycolysis is present in a region of recurrent, localized copy number gain at genomic locus 1p12. The amplification was most commonly found in melanoma, but was observed in other cancers, such as esophageal adenocarcinoma and triple-negative breast cancer. We validated this finding in a collection of human melanoma tissue samples, and it was observed that PHGDH protein expression correlated with genomic copy number gain.

Importantly, each melanoma cell line with PHGDH amplification exhibited significant flux into serine biosynthesis and required PHGDH expression for growth. Decreased expression of PHGDH in cells containing the amplification resulted in a distinct metabolic phenotype marked by accumulation of glycolytic intermediates. Little to no change in the citric acid cycle was observed. Since we observed no detectable flux in a non-tumorigenic breast epithelial cell line, we questioned whether enhanced PHGDH expression would have any phenotypic consequences. We considered the effects of PHGDH in a model of breast tissue morphogenesis.[8] In this model, ectopic expression of PHGDH increased pathway flux and induced loss of cell polarity, disruptions in nuclear architecture and rescue of cell death induced by matrix detachment. Each of these characteristics constitutes a phenotypic alteration that confers a predilection toward tumorgenicity, establishing *PHGDH* as a gene with oncogenic properties. These findings show that PHGDH could be a target for subsets of human cancers. As a metabolic enzyme with many small molecule binding sites and already-reported[9] non-selective inhibitors, such as 3-bromopyruvate, selective PHGDH-targeting agents should be feasible.

Gene amplification is one mechanism to enhance the rate at which glucose is diverted to de novo serine metabolism. Other mechanisms that increase PHGDH protein expression and activity undoubtedly exist. The transcriptional, translational and post-translational regulatory networks that control PHGDH protein levels have not yet been identified. It is likely that multiple oncogene and tumor suppressor networks regulate PHGDH expression. Flux through the serine biosynthetic pathway might be required for cancer growth in certain defined genetic contexts, and testing this hypothesis will require additional studies. In addition to mechanisms that enhance flux by increasing protein expression, other ways to regulate flux also exist. A recently identified feedback loop in glycolysis[10] involving the phosphorylation and priming of phosphoglycerate mutase might function to in part to direct glycolytic flux toward the 3PG step in glycolysis. Computational modeling studies predict that an enhanced flux to serine might be one consequence of priming PGAM in this manner (Locasale et al., in preparation).

The advantages that enhanced pathway flux confers to tumorigenic cells are many, multifactorial and likely to be different in different environments. Directing glycolytic intermediates into de novo serine biosynthesis allows for glucose to be utilized for numerous biosynthetic processes. These include the synthesis of nucleotides, proteins, lipids as well as glutathione, which has an intracellular concentration well over 1 mM. Each of the numerous anabolic pathways that utilize serine and glycine could have beneficial effects on cell growth. In addition to these effects, enhanced flux from glucose to serine exerts effects on the flux backbone
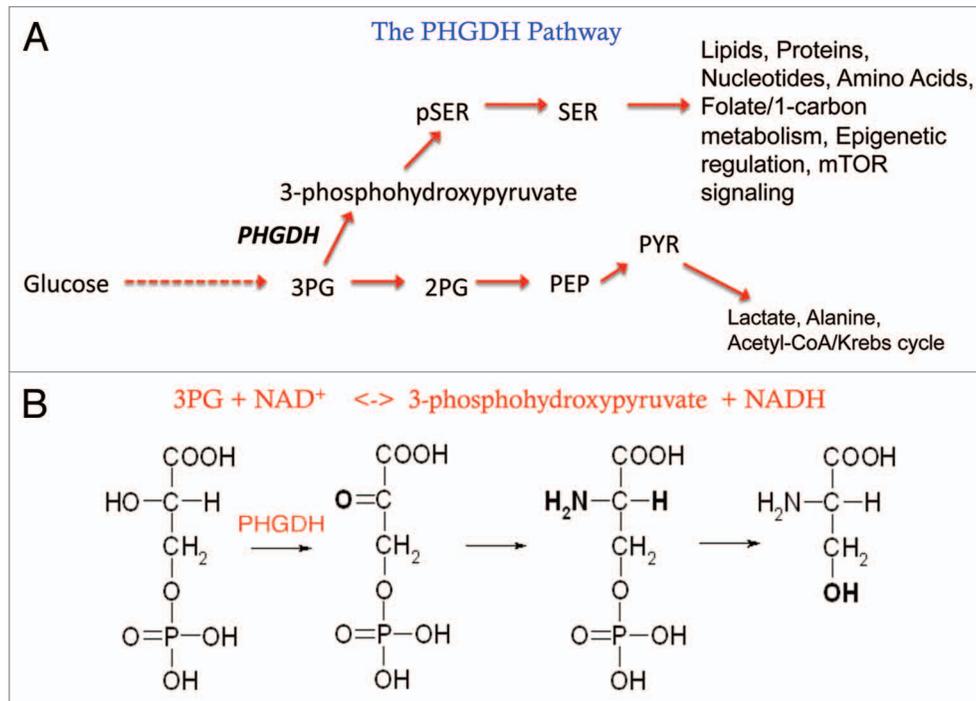
Figure 1. Diversion of glycolytic flux into de novo serine metabolism by PHGDH. (A) The PHGDH pathway. Glucose that is taken up and captured in cells is metabolized through a series metabolic reactions eventually leading to the generation of 3-phosphoglycerate (3PG). At this stage, there exists a branch point in glycolysis. Glucose can continue along the direction of glycolysis and eventually generate pyruvate and products of pyruvate such as alanine, lactate and acetyl-coA. (B) Alternatively, 3PG can by oxidized by PHGDH eventually leading to serine biosynthesis. Serine metabolism can result in the generation of lipids, proteins and nucleic acids. Serine metabolism also has signaling functions. The PHGDH branch point is upregulated in cancer cells, and evidence is presented that a genetic selection for altered metabolic flux through this branch exists in cancer development.

around glycolysis. This differential regulation of the glycolytic flux backbone is not captured by merely an enhanced uptake of serine from the environment. This change in the glycolytic flux backbone causes other metabolic fluxes emanating from glucose to be substantially altered. Such a change could manifest in different movements of carbon to pathways branching from glycolysis, such as the pentose phosphate pathway and glycerolipid synthesis pathway.

Diverting glucose out of glycolysis into serine synthesis also affects the fluxes derived from the coupled chemical reactions that occur along the way. The oxidation of 3PG generates NADH from NAD^+, and this reaction affects the overall redox balance in cells. This change in the NAD^+/NADH ratio affects signal transduction through changes in the activities of NAD-dependent enzymes, such as sirtuins or ADP-ribosyltransferases. Changes in the NAD^+/NADH ratio also affects the relative magnitudes of reductive fluxes

involved in biosynthesis. Flux through the PHDGH pathway via the transamination reaction generates α-ketoglutarate (αKG), which was observed to fuel the citric acid cycle in a recent study.[11] However, this effect is not likely to be general, since transamination via serine was not observed to be the predominant route to αKG in the cell lines examined in our study, and other transamination reactions such as pyruvate to alanine synthesis are known to be very active in many cancer cells. Thus, enhancing glycolytic flux through the PHGDH pathway, as is true with all known oncogenic events, exerts a multitude of effects on cellular physiology. It is likely that the combination of many of these effects in concert promotes tumorigenesis, and that a single mechanism in isolation is not likely to contribute to cell transformation in itself. Nevertheless, the observation that a genetic aberration selected for in tumor development functions to deregulate central carbon metabolism provides multiples avenues for future mechanistic studies.

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