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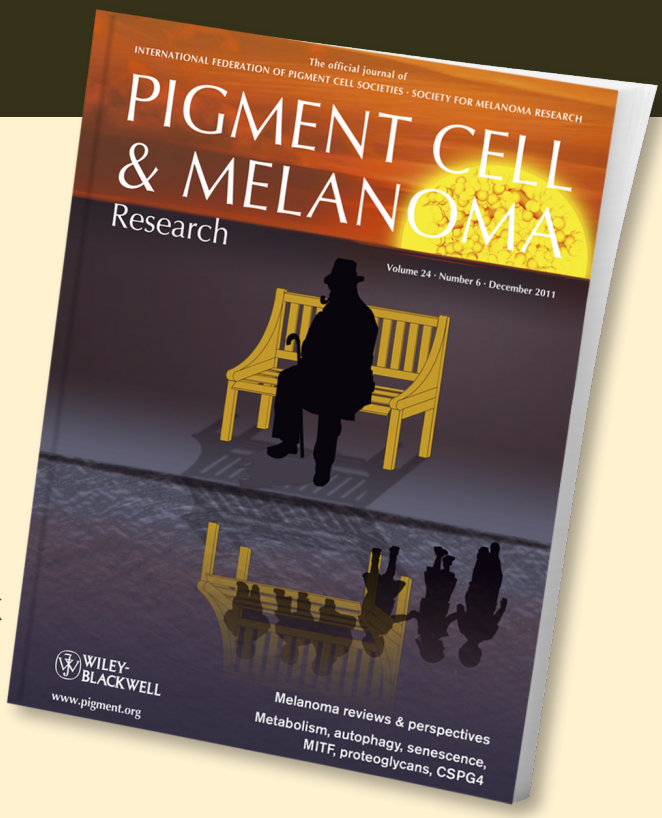

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PHGDH amplification and altered glucose metabolism in human melanoma

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Summary

The metabolic requirements of cancer cells differ from that of their normal counterparts. To support their proliferation, cancer cells switch to a fermentative metabolism that is thought to support biomass production. Instances where metabolic enzymes promote tumorigenesis remain rare. However, an enzyme involved in the de novo synthesis of serine, 3-phosphoglycerate dehydrogenase (PHGDH), was recently identified as a putative oncogene. The potential mechanisms by which PHGDH promotes cancer are discussed.

Since the seminal work of Otto Warburg, cancer cells have been known to import and consume increased amounts of glucose compared with most normal cells and to produce lactate even in the presence of oxygen. This switch to a fermentative metabolism is known as the Warburg effect (Warburg, 1924; Warburg, 1956). Why cancer cells engage in aerobic glycolysis despite the low ATP yield compared to the Krebs cycle is not clearly understood. One explanation could be that cancer cell proliferation is not limited by ATP production, but rather by the ability of cells to synthesize the lipids, nucleic acids, and proteins needed to produce biomass (Vander Heiden et al., 2009; Locasale et al., 2009; Deberardinis et al., 2008a,b). This hypothesis is consistent with the observation that cancer cells express the M2 isoform of pyruvate kinase. Pyruvate kinase generates the ATP derived from glycolysis by catalyzing a phosphotransfer reaction from phosphoenolpyruvate to ADP. Cancer cells express the less active PKM2 isoform, while many cell types requiring large amounts of ATP express the more active PKM1 isoform. PKM2 expression, and therefore decreased pyruvate kinase activity, might allow for a buildup of glycolytic intermediates and their subsequent funneling into anabolic processes (Christofk et al., 2008).

Metabolic enzymes can play an important role in tumorigenesis, as evidenced by the predisposition of individuals with germline mutations in succinate dehydrogenase and fumarate hydratase to develop cancer (Hao et al., 2009; Pollard et al., 2003). Perhaps, the best evidence for metabolic enzymes playing a causative role comes from the discovery of IDH1 and IDH2 mutations in several cancer types (Yen et al., 2010). Both IDH1 and IDH2, the cytosolic and mitochondrial forms of $NADP^+$-dependent isocitrate dehydrogenase, respectively, are subject to gain-of-function mutations whereby the enzymes produce 2-hydroxyglutarate instead of α-ketoglutarate (αKG) (Dang et al., 2009). Mutations in *IDH1* were found in acute myeloid leukemias and glioma. Strikingly, more than 70% of stages II–III astrocytoma or oligodendroglioma tumors had mutations in either *IDH1* or *IDH2* (Mardis et al., 2009; Thompson, 2009). Recent studies showed that 2-hydroxyglutarate can function as a competitive inhibitor of αKG-dependent dioxygenases, including histone demethylases and the TET (Ten-Eleven-Translocation) family of 5-methylcytosine hydroxylases, suggesting one possible mechanism for how 2-hydroglutarate functions as an oncometabolite (Figueroa et al., 2010; Xu et al., 2011). In support of the plausibility of this mechanism, TET2 mutations that abrogate catalytic activity have been found in myeloid malignancies, promote myeloid tumorigenesis, and are mutually exclusive with IDH1 and IDH2 mutations (Ko et al., 2010, 2011; Moran-Crusio et al., 2011). Despite the preceding example, instances where metabolic enzymes are mutated to promote tumorigenesis have not been described.

Now, it has been found that an increased expression of PHGDH – the first enzyme in the serine biosynthesis pathway originating from the glycolytic intermediate 3-phosphoglycerate – is linked to tumorigenesis (Locasale et al., 2011). The serine biosynthesis pathway or PHGDH pathway consists of three sequential enzymatic reactions: (i) phosphoglycerate dehydrogenase (PHGDH) oxidizes phosphoglycerate (3PG) to 3-phosphohydroxypyruvate (pPYR); (ii) phosphoserine amino transferase (PSAT) transaminates pPYR to phosphoserine (pSER) using glutamate (GLU) as the nitrogen donor; (iii) phosphoserine phosphatase dephosphorylates (PSPH) pSER to produce serine in an essentially irreversible reaction (Figure 1). It is noteworthy that Snell and coworkers had previously identified enhanced activity of the PHGDH pathway in homogenates of neoplastic tissues relative to normal tissues (Snell, 1984, 1986). However, the necessary and sufficient conditions for this pathway to promote tumorigenesis have not been previously characterized.

New evidence suggests that *PHGDH*, the gene encoding the enzyme that controls flux from glycolysis into the serine biosynthesis pathway, is a candidate oncogene. *PHGDH* was found to be frequently amplified in a pooled analysis of somatic copy number alterations across 3131 human cancer samples (Beroukhim et al., 2010). Furthermore, *PHGDH* amplifications were focal in some tumors, and no known oncogenes were contained within the focal amplification peak. Melanoma, with 39% of samples exhibiting some form of copy number gain, had the highest frequency of *PHGDH* amplification among tumor types included in the analysis (Figure 2A). *PHGDH* expression was confirmed experimentally in human samples (Figure 2B). Consistent with the genetic analysis, knockdown of PHGDH inhibited the growth of melanoma

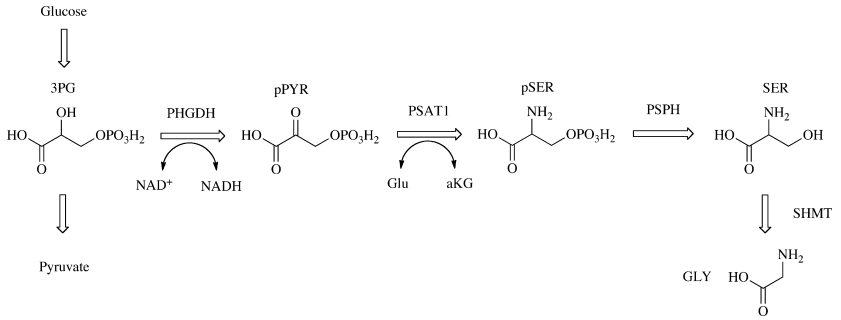

Figure 1. The PHGDH pathway. The pathway begins with the oxidation of the glycolytic intermediate 3PG to 3-phosphohydroxypyruvate (pPYR) with the concomitant reduction of the NAD^+ cofactor to NADH by the enzyme PHGDH. PSAT1 then uses the α-amino group of glutamate (GLU) to transaminate pPYR yielding phosphoserine (pSER) and α-ketoglutarate (αKG). Finally, phosphoserine phosphatase (PSPH) dephosphorylates pSER to give SER. PHGDH, phosphoglycerate dehydrogenase; SHMT, serine hydroxymethyltransferase; GLY, glycine.

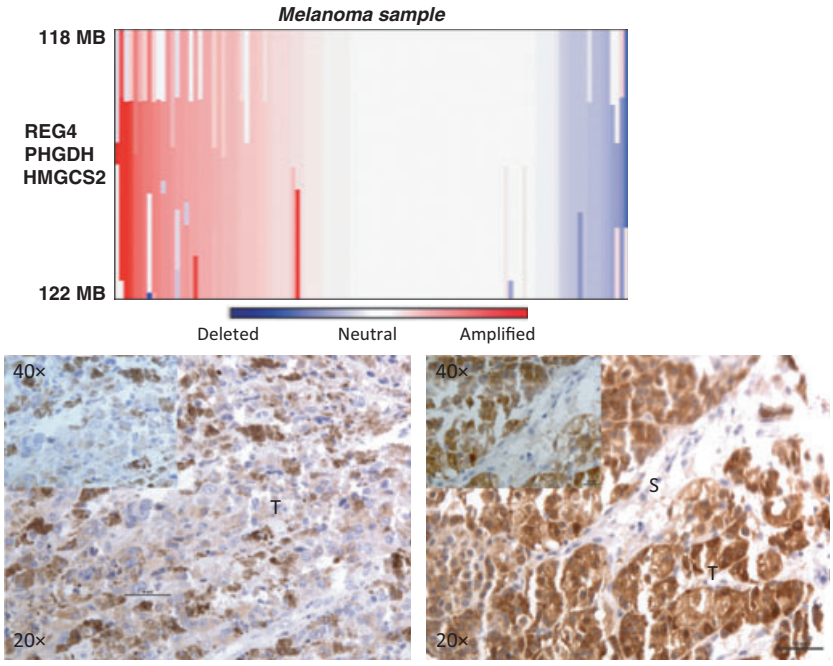

Figure 2. *PHGDH* is amplified and expressed in melanoma. (A) Melanoma samples (n = 150) sorted by intensity of the *PHGDH* genomic locus at 1p12. Copy number gain was found with a frequency of 39%. (B) Immunohistochemistry (IHC) of 2 representative human tissue samples. *PHGDH* is expressed in the tumor (T) but not in the stroma (S). PHGDH, phosphoglycerate dehydrogenase.

cell lines that harbored the *PHGDH* amplification but had no effect on lines lacking the amplification.

Further phenotypic consequences of enhanced PHGDH expression can be seen in MCF-10A cells, a human non-transformed mammary epithelial cell line. In reconstituted basement membrane, MCF-10A cells form polarized, growth-arrested, acini-like spheroids that mimic in vivo glandular architecture (Debnath and Brugge, 2005; Debnath et al., 2003). The acini grow from single cells into spherical structures that hollow out as the luminal cells deprived of contact with the basement membrane undergo apoptosis. Surprisingly, upon *PHGDH* overexpression, luminal cells fail to undergo apoptosis when detached from the extracellular matrix.(Locasale et al., 2011). Moreover, the acini show excessive proliferation and disrupted polarity. These phenotypes are suggestive of a predisposition for transformation and are even reminiscent of those seen with overexpression of the canonical breast cancer oncogene *ERBB2*.

Possemato et al. (2011) reported that *PHGDH* knockdown in breast cancer MDA-MB-468 cells causes αKG levels and other TCA cycle intermediates to drop without affecting serine levels and that PSAT activity is responsible for a large fraction of the net conversion of GLU to αKG. Previously, it had been reported that glutamate dehydrogenase or alanine or aspartate transaminase was major contributor to αKG-mediated anaplerosis; it is therefore surprising that PSAT was found to be a strong modulator of αKG levels (Deberardinis and Cheng, 2010). However, this effect is not likely to be general because knockdown of PHGDH in many amplified cell lines that are sensitive to PHGDH knockdown did not result in a decrease in the levels of TCA intermediates (Locasale et al., 2011). In addition, alanine rather than serine is the major amino acid nitrogen acceptor from GLU in multiple cell lines containing *PHGDH* amplification (Locasale et al., 2011). Taken together, these data suggest that there are other growth-mediating advantages to acquiring *PHGDH* amplification.

It is surprising that cells grown in standard tissue culture conditions, where extracellular serine is abundant, are sensitive to *PHGDH* knockdown. One wonders why cells are unable to grow without PHGDH when serine is readily available. There are multiple feasible hypotheses, including the possibility that the cells might not express the correct amino acid transporters to import enough serine. Also, the homeostatic mechanisms that balance and regulate metabolic flux are not necessarily coupled to the extra- and intracellular serine pools. Furthermore, obtaining serine via a de novo biosynthesis pathway differs from the import of serine, because de novo synthesis involves the flow of carbon through glycolysis and all pathways branching from it, including but not limited to serine metabolism. Flux through PHGDH and the serine biosynthetic pathway has effects aside from serine production. The PHGDH reaction involves the oxidation of 3PG, potentially affecting the NAD^+/NADH ratio and cellular redox balance. In addition, the second reaction in the pathway catalyzed by PSAT is a transamination reaction utilizing pPYR and GLU to produce pSER and αKG. αKG is used in a plethora of other biochemical reactions.

How enhanced flux through PHGDH promotes and is required for tumorigenesis remains unclear. Serine is an important precursor for biomass production: It is an abundant constituent of proteins and is essential for the synthesis of sphingolipids and most phospholipid head groups, which are important components of cellular membranes and are also involved in cell signaling (Futerman and Riezman, 2005; Kuge and Nishijima, 2003). Serine hydroxymethyltransferase catalyzes the conversion of serine to glycine with the concomitant conversion of tetrahydrofolate (THF) to 5,10-methylene-THF. This reaction is an important source of one carbon (1C) units for the cellular THF pool. By contributing 1C units to the folate pool and de novo synthesis of glycine, serine provides carbon skeletons for the synthesis of purines and pyridines (De Koning et al., 2003; Pind et al., 2002). The numerous contributions of PHGDH to biomass production may explain part of its pro-tumorigenic effect.

In cells with large fluxes through PHGDH, generating αKG through the serine biosynthesis pathway may alleviate ammonia toxicity that may occur as a result of amino acid catabolism. For example, when GLU derived from glutamine is catabolized by glutamate dehydrogenase to eventually derive αKG, a unit of ammonia is produced and this ammonia production is alternatively bypassed by αKG synthesis via the serine or alanine synthesis pathway. Ammonia levels have been shown to regulate autophagy, and it would be interesting to see whether cells that require PHGDH have defects in autophagy after PHGDH knockdown (Cheong et al., 2011; Eng et al., 2010).

Cellular redox status is in part determined by the NAD^+/NADH ratio. Diverting glycolytic flux into serine synthesis produces twice as much cytosolic NADH per glucose when compared with conventional glycolysis to generate pyruvate, and thus, it is possible that decreased NADH production resulting from PHGDH knockdown induces redox stress. As less pyruvate (the substrate of lactate dehydrogenase, which normally regenerates NAD^+) is produced when flux is diverted into the serine biosynthesis pathway, cells with elevated serine biosynthesis also need to regenerate more NAD^+ to maintain high rates of glycolysis. How cells regenerate NADH back to NAD^+ in *PHGDH*-amplified cells is unclear. One possibility is that the glycerol phosphate shuttle, which ultimately transfers electrons from cytosolic NADH to FAD^+ on mitochondrial complex II, may provide one mechanism. If so, this pathway could provide an additional benefit by allowing mitochondrial ATP synthesis with production of less ROS by passing the electrons from NADH to complex II as opposed to mitochondrial complex I.

Much of the renewed interest in studying metabolism has focused on glycolysis, the citric acid cycle, and glutamine metabolism. Although central to the functioning of a cell, these pathways are only part of the larger metabolic network, and other metabolic alterations in cancer cells remain poorly characterized. The oncogenic nature of *PHGDH* amplification likely stems from a combinatorial effect of pathway flux toward biomass production, changes in redox status, energy metabolism, and possibly some signaling functions. Additionally, the benefit tumors acquire from amplifying *PHGDH* likely varies based on environmental factors, tissue of origin, and cooperating oncogenic mutations. Mapping the contextual dependencies for the necessary and sufficient metabolic pathway alterations occurring in cell proliferation and transformation will expand our understanding of how altered metabolism contributes to disease.

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References

Beroukhim, R., Mermel, C.H., Porter, D. et al. (2010). The landscape of somatic copy-number alteration across human cancers. Nature *463*, 899–905.

Cheong, H., Lindsten, T., Wu, J., Lu, C., and Thompson, C.B. (2011). Ammonia-induced autophagy is independent of ULK1/ULK2 kinases. Proc. Natl Acad. Sci. USA *108*, 11121–11126.

Christofk, H.R., Vander Heiden, M.G., Harris, M.H., Ramanathan, A., Gerszten, R.E., Wei, R., Fleming, M.D., Schreiber, S.L., and Cantley, L.C. (2008). The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. Nature *452*, 230–233.

Dang, L., White, D.W., Gross, S. et al. (2009). Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature *462*, 739–744.

De Koning, T.J., Snell, K., Duran, M., Berger, R., Poll-The, B.T., and Surtees, R. (2003). L-serine in disease and development. Biochem. J. *371*, 653–661.

Deberardinis, R.J., and Cheng, T. (2010). Q's next: the diverse functions of glutamine in metabolism, cell biology and cancer. Oncogene *29*, 313–324.

Deberardinis, R.J., Lum, J.J., Hatzivassiliou, G., and Thompson, C.B. (2008a). The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell Metab. *7*, 11–20.

Deberardinis, R.J., Sayed, N., Ditsworth, D., and Thompson, C.B. (2008b). Brick by brick: metabolism and tumor cell growth. Curr. Opin. Genet. Dev. *18*, 54–61.

Debnath, J., and Brugge, J.S. (2005). Modelling glandular epithelial cancers in three-dimensional cultures. Nat. Rev. Cancer *5*, 675–688.

Debnath, J., Muthuswamy, S.K., and Brugge, J.S. (2003). Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures. Methods *30*, 256–268.

Eng, C.H., Yu, K., Lucas, J., White, E., and Abraham, R.T. (2010). Ammonia derived from glutaminolysis is a diffusible regulator of autophagy. Sci. Signal. *3*, ra31.

Figueroa, M.E., Abdel-Wahab, O., Lu, C. et al. (2010). Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. Cancer Cell *18*, 553–567.

Futerman, A.H., and Riezman, H. (2005). The ins and outs of sphingolipid synthesis. Trends Cell Biol. *15*, 312–318.

Hao, H.X., Khalimonchuk, O., Schraders, M. et al. (2009). SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. Science *325*, 1139–1142.

Ko, M., Huang, Y., Jankowska, A.M. et al. (2010). Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. Nature *468*, 839–843.

Ko, M., Bandukwala, H.S., An, J., Lamperti, E.D., Thompson, E.C., Hastie, R., Tsangaratou, A., Rajewsky, K., Koralov, S.B., and Rao, A. (2011). Ten-Eleven-Translocation 2 (TET2) negatively regulates homeostasis and differentiation of hematopoietic stem cells in mice. Proc. Natl Acad. Sci. USA *108*, 14566–14571.

Kuge, O., and Nishijima, M. (2003). Biosynthetic regulation and intracellular transport of phosphatidylserine in mammalian cells. J. Biochem. *133*, 397–403.

Locasale, J.W., Cantley, L.C., and Vander Heiden, M.G. (2009). Cancer's insatiable appetite. Nat. Biotechnol. *27*, 916–917.

Locasale, J.W., Grassian, A.R., Melman, T. et al. (2011). Phosphoglycerate dehydrogenase diverts glycolytic flux and contributes to oncogenesis. Nat. Genet. *43*, 869–874.

Mardis, E.R., Ding, L., Dooling, D.J. et al. (2009). Recurring mutations found by sequencing an acute myeloid leukemia genome. N. Engl. J. Med. *361*, 1058–1066.

Moran-Crusio, K., Reavie, L., Shih, A. et al. (2011). Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. Cancer Cell *20*, 11–24.

Pind, S., Slominski, E., Mauthe, J., Pearlman, K., Swoboda, K.J., Wilkins, J.A., Sauder, P., and Natowicz, M.R. (2002). V490M, a common mutation in 3-phosphoglycerate dehydrogenase deficiency, causes enzyme deficiency by decreasing the yield of mature enzyme. J. Biol. Chem. *277*, 7136–7143.

Pollard, P.J., Wortham, N.C., and Tomlinson, I.P. (2003). The TCA cycle and tumorigenesis: the examples of fumarate hydratase and succinate dehydrogenase. Ann. Med. *35*, 632–639.

Possemato, R., Marks, K.M., Shaul, D.Y. et al. (2011). Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. Nature *476*, 346–350.

Snell, K. (1984). Enzymes of serine metabolism in normal, developing and neoplastic rat tissues. Adv. Enzyme Regul. *22*, 325–400.

Snell, K. (1986). The duality of pathways for serine biosynthesis is a fallacy. Trends Biochem. Sci. *11*, 241–243.

Thompson, C.B. (2009). Metabolic enzymes as oncogenes or tumor suppressors. N. Engl. J. Med. *360*, 813–815.

Vander Heiden, M.G., Cantley, L.C., and Thompson, C.B. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science *324*, 1029–1033.

Warburg, O. (1956). On the origin of cancer cells. Science *123*, 309–314.

Warburg, O., Posener, K., and Negelein, E. (1924). Ueber den Stoffwechsel der Tumoren. Biochem. Z. *152*, 319–344.

Xu, W., Yang, H., Liu, Y. et al. (2011). Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. Cancer Cell *19*, 17–30.

Yen, K.E., Bittinger, M.A., Su, S.M., and Fantin, V.R. (2010). Cancer-associated IDH mutations: biomarker and therapeutic opportunities. Oncogene *29*, 6409–6417.