

## IN THE SPOTLIGHT

## Glycolysis Back in the Limelight: Systemic Targeting of HK2 Blocks Tumor Growth

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**Summary:** In a recent issue of *Cancer Cell*, Patra and colleagues report findings that underscore the importance of Hexokinase 2 (HK2) in tumor initiation and maintenance. The authors also show that HK2 can be systemically deleted without adverse physiologic consequences. These findings provide attractive insights into HK2 deletion as a potential therapeutic intervention for cancer. *Cancer Discov*; 3(10); 1105-7. ©2013 AACR.

A key hallmark of cancer cells is their capacity to metabolize glucose at an elevated rate (1, 2). Critical to this highly glycolytic phenotype is the first enzymatic step of glycolysis, catalyzed by hexokinase (HK), during which glucose is phosphorylated and thereby trapped within the cell. There are four HK enzymes in mammals, HK1, HK2, HK3, and glucokinase (GCK), which are structurally similar but expressed in a tissue-specific manner (3). Remarkably, only HK2 is overexpressed in cancer cells (4) and contributes to the high glycolytic rate in tumors (5). Given the selective expression of HK2 in cancer, Patra and colleagues (6) investigated whether targeting this particular glycolytic enzyme could be used for cancer therapy.

Several previous studies have reported that inhibition of HK2 can block cancer cell survival. Depletion of HK2 with short hairpin RNA (shRNA) inhibited tumor growth in a xenograft model of glioblastoma multiforme (7). Moreover, HK2 mRNA and protein levels were found to be upregulated in a derivative of the human breast carcinoma cell line MDA-MB-231 that metastasizes to the brain (8). HK2 knockdown using shRNA reduced cell proliferation and viability under conditions of limited glucose availability in these cells (8). The micro-RNA miR-143, which is downregulated in response to activation of the mTOR, has been shown to reduce glucose metabolism and inhibit lung cancer cell proliferation and tumor formation by targeting HK2, an effect that was reproduced by direct silencing of HK2 (9). However, the role of HK2 in tumor initiation and maintenance remained unclear. More importantly, it had to be shown that systemic inhibition of HK2 could be a viable approach to treating cancer.

Patra and colleagues (6) examined the role of HK2 in tumor initiation using a genetic model of non-small cell lung carcinoma (NSCLC) induced by expression of activated KRAS (KRAS-LA2) and a model of breast cancer induced by expres-

sion of activated ERBB2/Neu (MMTV-*neu*). They found that genetic deletion of *Hk2* reduced overall tumor burden in lungs of *Kras*-mutant mice. This was caused by a reduction in the number rather than the size of individual lesions, indicating a role for HK2 in tumor initiation. In the breast cancer model, genetic deletion of *Hk2* extended the latency of tumor development, resulting in a substantial increase in survival. The study also addressed whether HK2 is also required for tumor maintenance in lung cancer. HK2 ablation, via inducible shRNA, reduced the growth of NSCLC cells in a xenograft model. Similar inhibition of proliferation was also observed in cancer cells derived from a genetic model of KRAS-driven lung cancer (KRAS-LA2) after genetic deletion of *Hk2*. The authors also show that deletion of *Hk2* in murine cancer cells derived from the MMTV-*neu* mouse model reduces their ability to form tumors after orthotopic implantation into athymic mice, again associated with reduced rates of proliferation. These results show a role for HK2 in tumor initiation and maintenance. However, it could also be interesting to address whether HK2 also plays a role in the progression to the metastatic state. Indeed, increased HK2 expression has been associated with poor patient survival in a cohort of 123 resected brain metastases of breast cancer (8).

Patra and colleagues (6) addressed the metabolic consequences of *Hk2* deletion in cancer cell lines derived from the KRAS-LA2 model. They found that *Hk2* deletion caused a reduction in the entry of metabolites into the serine biosynthesis pathway, reduced glucose- and glutamine-dependent fatty acid biosynthesis, and impaired nucleotide biosynthesis through the nonoxidative arm of the pentose phosphate pathway. They also found that *Hk2* deletion inhibited the entry of glutamine-derived metabolites into the tricarboxylic acid cycle (anaplerosis) but increased glutamine-derived production of citrate by reductive carboxylation of  $\alpha$ -ketoglutarate, thereby partially compensating for the reduction in glucose-derived citrate and acetyl-CoA for lipid synthesis (Fig. 1).

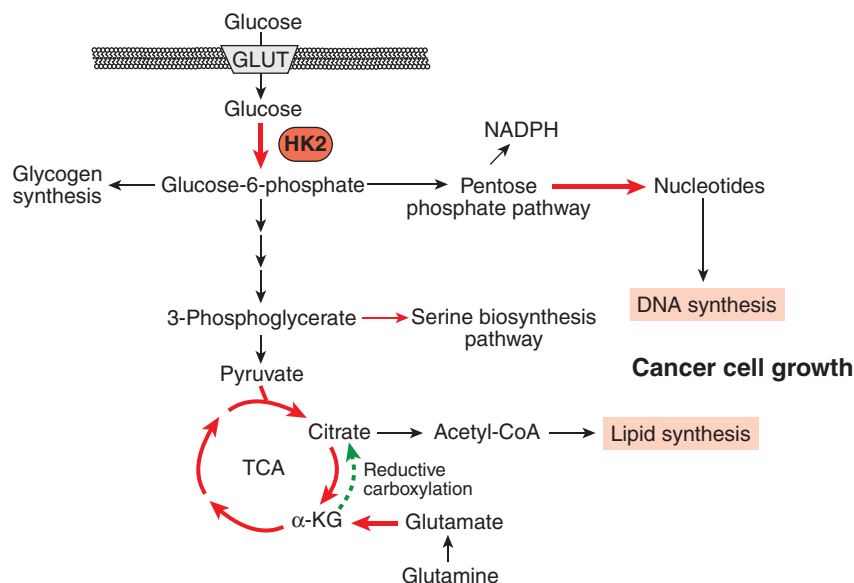
These results identify HK2 as a key player in the diversion of glucose-derived carbons into pathways required for anabolic processes in cancer cells and could explain why cancer cells express such high levels of this enzyme. But why do cancer cells preferentially use HK2 instead of other hexokinases? HK2 has high affinity for glucose and harbors two catalytic domains that have both retained their catalytic activity. In

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**Figure 1.** Remodeling of cancer metabolism by HK2. Schematic illustration of the proposed metabolic consequences induced by HK2 in cancer cells. Increased expression of HK2 in cancer cells drives nucleotide and lipid biosynthesis as well as glutamine-dependent anaplerosis. This supports anabolic metabolism, which is essential for tumor growth. Increased flux is depicted in red, decreased in green. TCA, tricarboxylic acid; GLUT, glucose transporter.

addition, HK2 has an N-terminal domain that allows it to bind to the mitochondrial outer membrane voltage-dependent anion channel (VDAC). Binding to VDAC enhances the affinity of hexokinases for ATP and makes them insensitive to inhibition by their product, glucose-6-phosphate (3). Mitochondrial HK2 also inhibits apoptosis by preventing the recruitment of proapoptotic proteins such as Bax (10), by regulating the mitochondrial permeability transition pore (11), and by limiting the production of reactive oxygen species (ROS; ref. 12). More recently, it has been shown that the Tp53-induced glycolysis and apoptosis regulator binds to and activates mitochondrial HK2 in hypoxic cells, which resulted in reduced levels of ROS and lowered cell death (13).

Mitochondrial association of HK2 couples glucose phosphorylation to oxidative metabolism and the control of apoptosis (14). It has previously been shown that HK2 binding to VDAC is promoted by activation of oncogenic signaling pathways, such as phosphoinositide 3-kinase (PI3K)/AKT activation (15). Interestingly, Patra and colleagues (6) now show that oncogenic *Ras* also increases the expression of HK2, and that both PI3K and MAP-ERK kinase (MAPK) signaling contribute to this induction, thereby adding an additional mode of regulation of metabolic activity in cancer cells.

Another important point addressed by Patra and colleagues (6) is whether systemic inhibition of HK2 is effective in blocking tumor growth without producing any adverse physiologic consequences. HK2 is expressed mostly in embryonic tissues and in adult adipose tissue as well as skeletal and cardiac muscle (3). To investigate the effect of systemic deletion, the authors deleted *Hk2* in the KRAS-LA2 model by using an inducible CRE recombinase at 2 months of age, when tumor initiation is likely to have already occurred. This late deletion of *Hk2* resulted in a reduction of tumor burden, with some of the residual lesions showing normal levels of *Hk2* expression, most likely due to incomplete excision. These observations suggest that systemic ablation of HK2 may be a way of selectively killing cancer cells without toxicity to healthy tissues.

The results shown by Patra and colleagues (6) provide evidence for the potential effectiveness of HK2-specific inhibitors. Current agents targeting HK2, like 3-bromopyruvate and 2-deoxyglucose, may have limited clinical potential due to low specificity. One possible strategy to improve selectivity would be to target HK2 binding to VDAC, thereby blocking its mitochondrial association and reducing its activity. In this context, it will be interesting to investigate whether inhibition of mitochondrial association will replicate the metabolic consequences of HK2 ablation observed in the current study. Selective inhibitors of HK2 could be used to treat specific patient populations, such as those showing mutations in oncogenic pathways that promote HK2 expression and activity. In addition, <sup>18</sup>F-Fludeoxyglucose-positron emission tomography could also be used for patient stratification. Finally, inhibition of HK2 could also be attractive for use in combination with chemotherapeutic drugs or targeted therapies.

In summary, Patra and colleagues (6) provide important insights into the role of HK2 in tumor initiation and maintenance as well as the metabolic consequences of HK2 depletion. While the feasibility of the systemic targeting of glycolytic enzymes for cancer therapy is not fully established, HK2 could be an attractive candidate for such intervention.

### Disclosure of Potential Conflicts of Interest

A. Schulze performs minor consultancy work for TPP Global Development. No potential conflicts of interest were disclosed by the other author.

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