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Targeting hexokinase 2 in castration-resistant prostate cancer

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Aerobic glycolysis, known as the Warburg effect, is one of the hallmarks of cancer cells. We recently reported that the hexokinase 2 (HK2)-mediated Warburg effect is required for castration-resistant prostate cancer that is driven by *Pten/p53* deficiency, suggesting that HK2 might be a therapeutic target for prostate cancer patients carrying *PTEN* and *p53* mutations.

Keywords: CRPC, HK2, PTEN, p53, Warburg effect

Abbreviations: 2-DG, 2-deoxyglucose; 3-BrpA, 3-bromopyruvate; 4E-BP1, eIF4E-binding protein 1; CRPC, castration-resistant prostate cancer; eIF4E, eukaryotic translation initiation factor 4E; FDG, fluorodeoxyglucose; G-6-P, glucose-6-phosphate; HK, hexokinase; mTORC1, mammalian target of rapamycin complex 1; p53, tumor suppression protein 53; PET, positron emission tomography; PTEN, phosphatase and tensin homolog.

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Accelerated glucose metabolism in cancer cells under aerobic conditions, a phenomenon known as the Warburg effect, leads to high uptake of the labeled glucose analog fluorodeoxyglucose (FDG), which can be clinically useful to detect tumors and monitor therapeutic responses of cancer patients by positron emission tomography (PET).[1] Thus, identification of the enzyme(s) that catalyze the elevated glucose metabolism in cancer cells could be exploited not only to distinguish cancer cells from normal cells, but also to preferentially target cancer cells while sparing healthy cells.

Hexokinases (HKs) catalyze the essentially irreversible first step of glucose metabolism in cells by phosphorylating glucose to glucose-6-phosphate (G-6-P). There are 4 HK isoforms encoded by separate genes, HK1, HK2, HK3, and HK4 (also known as glucokinase). HK1 is ubiquitously expressed in almost all mammalian tissues and HK2 is normally expressed in insulin-sensitive tissues such as adipose, skeletal, and cardiac muscles. HK3 is usually expressed at low levels and HK4 expression is restricted to the pancreas and liver.[2]

Although elevated HK2 expression has been observed in certain types of cancer cell and in tumor tissues from mouse models and/or human patients,[3,4] the molecular mechanisms underlying HK2 upregulation remain incompletely understood. Accumulating evidence suggests that co-deletion of tumor suppressor genes, such as phosphatase and tensin homolog (*PTEN*) and tumor suppressor protein *p53* (*TP53*, best known as *p53*), plays a crucial role in the development of castration-resistant prostate cancer (CRPC) *in vivo*.[5] Through integrated analyses of mouse embryonic fibroblasts (MEFs) deficient in *Pten* and *p53*, prostate cancer cell lines, xenografts, and genetically engineered mouse models (GEMMs), as well as clinic prostate cancer samples, we have found that *Pten*/*p53* deficiency selectively enhances expression of HK2, but not HK1, through post-transcriptional and translational regulation.[6] Regarding the underlying mechanism, we have demonstrated that activation of AKT–mTORC1 signaling as a result of *Pten* deletion increases HK2 expression primarily at the translational level through phosphorylation of eIF4E-binding protein 1 (4E-BP1), whereas loss of *p53* decreases the biogenesis of miR143, which in turn causes degradation of *HK2* mRNA. As a result, the combined deficiency of *PTEN* and *p53* in prostate cancer cells synergistically leads to robustly elevated HK2 expression (**Fig. 1**; ref. 6).

Notably, HK2 is almost exclusively expressed in human prostate cancer tissue compared with normal prostate tissue, and its expression is particularly elevated in human prostate cancer harboring *PTEN*/*p53* mutations.[6] In line with our findings that HK2 expression level positively correlates with the Gleason score, the sensitivity and positive predictive value of FDG-PET based on HK2-mediated phosphorylation of FDG for detecting

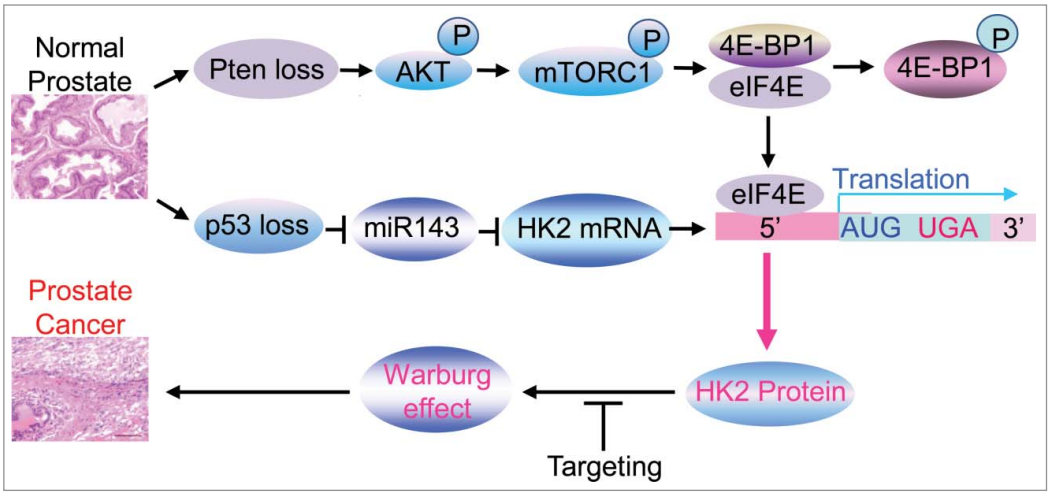

Figure 1. Induction of the HK2-mediated Warburg effect is required for *PTEN/p53* deficiency-driven prostate tumorigenesis. Loss of *PTEN* in prostate epithelial cells activates AKT–mTORC1 signaling to initiate phosphorylation of 4E-BP1, which releases eIF4E allowing formation of the translation initiation complex at the 5′ end of HK2 mRNA, and prompting cap-dependent translation. Loss of *p53* in prostate epithelial cells decreases the biogenesis of miR143, which in turn leads to the degradation of *HK2* mRNA. Accordingly, co-deletion of *PTEN* and *p53* robustly increases HK2 protein expression. The overexpressed HK2 protein initiates Warburg effect-dependent prostate tumor growth; therefore, targeting HK2 may inhibit prostate tumorigenesis that is driven by *PTEN/p53* deficiency. 4E-BP1, eIF4E-binding protein 1; eIF4E, eukaryotic translation initiation factor 4E; mTORC1, mammalian target of rapamycin complex 1.

patients with advanced prostate cancers was as high as 87%.[7] These results imply that HK2 expression distinguishes cancer cells from normal cells and could serve as a potential diagnostic and prognostic biomarker for advanced human prostate cancer, especially for patients harboring defects or mutations in *PTEN* and *p53*. Our genetic studies demonstrated that the HK2-mediated Warburg effect is required for the growth of *Pten/p53*-deficient prostate cancer cells *in vitro* and in xenograft models carrying mouse or human *PTEN/p53*-deficient prostate cancer cell lines *in vivo*.[6] These findings are consistent with a previous study of glioblastoma showing that HK2 depletion by shRNA inhibits tumor growth in a xenograft model.[4] More recently, a study using *Hk2* conditional knockout mice found that HK2 is required for tumor initiation and maintenance in mouse models of *Kras*-driven lung cancer and *ErbB2*-driven breast cancer.[3] Our study extends the biological significance of HK2 to prostate cancers carrying *Pten/p53* mutations, which drive the genesis of currently incurable castration-resistant prostate cancer (CRPC).[8]

Systemic deletion of *Hk2* in genetic mouse models inhibits tumor progression but does not impair normal glucose homeostasis or elicit any notable phenotypes *in vivo*,[3] indicating that HK2 could be a selective therapeutic target for cancer without any adverse physiological consequences. Given that our genetic findings support the crucial role of increased HK2 expression in driving the Warburg effect and prostate tumor progression in the presence of physiological levels of HK1, selectively targeting HK2 in these prostate cancer cells could be exploited as a promising personalized therapeutic strategy for patients with CRPC carrying *Pten/p53* mutations (**Fig. 1**). However, it would be very challenging to design an isoform-specific pharmacological inhibitor because HK2 has overlapping enzymatic activities with the ubiquitously expressed HK1, which is required for glucose metabolism of normal cells. Considering that only the C-terminal half of HK1 retains catalytic activity, whereas both N- and C-terminal halves of HK2 are catalytically active with the N-terminal half showing higher enzyme activity,[9] it is possible to use computational bioinformatics to identify small molecular compounds that might specifically block HK2 activity by targeting the N-terminal half of HK2 in cancer cells. In addition, the enzymatic activities of HK1 and HK2 are inhibited to the same extent by their own product G-6-P, yet inorganic phosphate prevents the inhibition of HK1 by G-6-P while enhancing the inhibition of HK2.[2] Thus, a G-6-P mimetic may preferentially inhibit HK2 activity in cancer cells.

Several critical questions arise from our findings. Does genetic deficiency of *Hk2* (conditional deletion of *Hk2* in prostate epithelial cells) effectively inhibit *Pten/p53* deficiency-driven CRPC in genetically engineered "triple-deficient" mouse models? Can currently available HK2 enzymatic inhibitors such as 2-deoxyglucoe (2-DG) and 3-bromopyruvate (3-BrpA) inhibit *Pten/p53* deficiency-driven CRPC *in vivo*? Does AKT–mTORC1–4EBP1-mediated translation signaling contribute to HK2 overexpression in prostate cancer cells carrying alterations in genes other than *Pten* and *p53*? Addressing these questions will provide deeper insights into the regulation and crucial role of HK2 in prostate tumorigenesis and potentially open up new avenues to treat currently incurable CRPC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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