MOLECULAR & CELLULAR ONCOLOGY
2019, VOL. 6, NO. 5, e1644599 (2 pages)
https://doi.org/10.1080/23723556.2019.1644599

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AUTHOR'S VIEW

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HectH9 hijacks glucose metabolism to fuel tumor growth

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ABSTRACT

Our study uncovered that HectH9 drives glycolysis and tumor development by K63-linked ubiquitination of Hexokinase 2 (HK2). This mechanism is critical for HK2 localization to mitochondria for activating HK2's functions in glycolysis promotion and apoptosis inhibition, suggesting that targeting HectH9 is a new strategy to tackle metabolism-addicted tumors.

ARTICLE HISTORY
Received 28 June 2019
Revised 3 July 2019
Accepted 14 July 2019

KEYWORDS
Cancer metabolism; ubiquitination; hexokinase; HectH9

Ubiquitination is a dynamic posttranslational modification originally known to mark protein for proteasomal degradation. Aside from proteolysis, ubiquitination regulates a variety of cellular processes, which include protein trafficking, signal transduction, gene transcription, and membrane protein sorting for recycling or lysosomal degradation. Dysregulated ubiquitination is known to drive the development of various human diseases, including cancer. Ubiquitin ligases account for substrate recognition in the ubiquitination machinery. Cancer-relevant ubiquitin ligases have been regarded as attractive target for therapeutic interventions.[1,2] HectH9, also known as HUWE1, ARF-BP1 and MULE, is a HECT domain-containing ubiquitin ligase that catalyzes K48 and K63-linked polyubiquitination. HectH9 is frequently overexpressed in the tumors of prostate, breast, lung and pancreas, but found to be downregulated in stomach and brain cancers. The disparate oncogenic and tumor suppressive roles of HectH9 is largely attributed to the diverse downstream substrates that HectH9 interacts with. For instance, HectH9 promotes tumorigenesis by K63-linked ubiquitination and subsequent activation of Myc oncogene and/or by K48-linked ubiquitination-mediated degradation of p53 tumor suppressor. On the contrary, HectH9 can induce tumor cell apoptosis by K48-linked ubiquitination and degradation of the antiapoptotic protein MCL1.[3] Thus, identifying novel substrates of HectH9 and their functional consequences in tumorigenesis will provide a road map for potential application of HectH9-targeted strategies.

Constitutive glucose uptake and lactate production are hallmarks of cancer.[4] Hypoxia is a pervasive feature in the tumor microenvironment responsible for reprograming metabolic pathways toward glycolysis. Hypoxia has been shown to induce the expression of HectH9 in cancer cells.[5] In our recent study, we sought to comprehensively understand whether and how HectH9 is engaged in the glucose metabolic processes in cancer. Through ^{13}C-glucose tracing and functional characterization, we found that HectH9 globally alters the abundance of multiple glycolytic intermediates, including glucose-6-phosphate.[6] Glucose-6-phosphate conversion from glucose is the first committed step of glucose metabolism. This process is catalyzed by hexokinases (HKs). HK2 is the cancer-associated HK isoform. HK2 expression is generally low in normal tissues but is robustly elevated in various tumor tissues, such as prostate, breast, lung and liver tumors. HK1 is the most ubiquitously expressed isoform in adult tissues and is regarded as a house keeping isoform that maintains the metabolic homeostasis. 2-Deoxyglucose (2-DG), metformin and 3-Bromopyruvate are most commonly reported HK2 inhibitors. These agents directly inhibit HK activity and display promising antitumor efficacy in preclinical models.[4,7] However, their effects are not restrict to HK2. To elucidate if HectH9 is a selective activator of HK2, we examined the crosstalk between HectH9 and HKs. Our ubiquitination assays revealed that HectH9 directly promotes K63-linked ubiquitination of HK2 and this ubiquitination event is selective for HK2 but not HK1. It is because that the K104 residue, one of the primary ubiquitination sites of HK2, is absent in HK1. The result suggests that HectH9-mediated HK2 ubiquitination may be exploited for selective inhibition of HK2.[6]

Our studies showed that HectH9-driven ubiquitination regulates HK2 localization to mitochondria.[6] Mitochondrial localization of HK2 not only gains access to the mitochondrial produced ATP for facilitating the glycolytic process, but also promotes the complex formation with the protein VDAC (voltage dependent anion channel) to prevent cellular apoptosis. We showed that genetic targeting of HectH9 concomitantly disrupts glycolysis and triggers apoptosis, in turn leading to the suppression of tumorigenesis. Our work uncovers that HectH9 is a bona fide regulator of HK2 and glucose metabolism. Myc is an established substrate of HectH9 and a critical modular of glycolysis. Our finding does not exclude the possibility that HectH9 promotes glycolysis in part through Myc. It's worth mentioning that our lactate production assays demonstrated that co-depletion of Myc and HectH9 led to greater glycolysis inhibition than single depletion of Myc. This finding indicates that HK2 is

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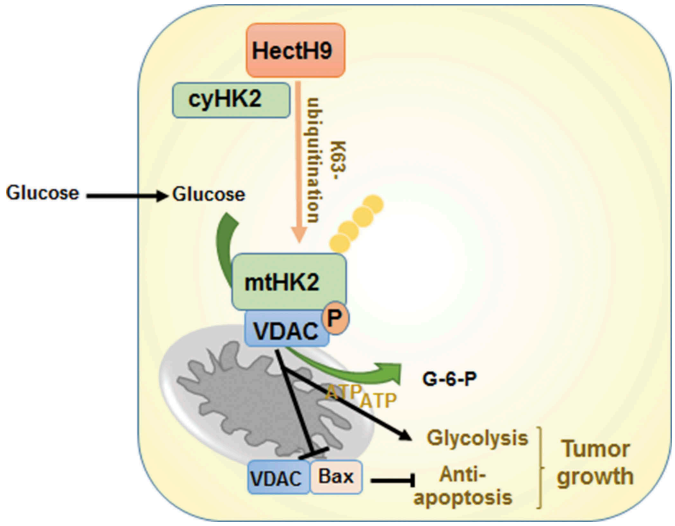

Figure 1. Molecular mechanisms through which HectH9 promotes tumor metabolism and growth.

The ubiquitin ligase HectH9 hijacks HK2 to mitochondria for accelerating HK2's dual functions in glycolysis promotion and apoptosis inhibition, which in turn lead to tumorigenesis. CyHK2 indicates the cytosolic HK2 while mtHK2 indicates the mitochondrial HK2. G-6-P refers to glucose-6-phosphate.

a critical downstream substrate underlying HectH9's profound effect on glucose metabolism besides Myc.

Mitochondrial HK2 is known to limit the production of reactive oxygen species (ROS). Our research demonstrated that the HectH9/HK2 pathway expands the cancer stem cell (CSC) population by ROS blockade, establishing a functional link between glycolysis modulators and cancer stemness. We further showed that ROS scavenging by N-acetylcysteine increases CSCs and counteracts the anti-cancer efficacy mediated by HectH9 or HK2 deficiency.[6] In line with our observation, recent animal studies showed that N-acetylcysteine and another antioxidant Vitamin E accelerates tumor progression in animal models.[8,9] These findings together cast caution to the use of antioxidants in cancer patients.

An earlier drug screen identified BI8626 is a HectH9-specific inhibitor.[10] We found that BI8626 profoundly inhibits the self-renewal of CSCs. We also demonstrated that 2-DG inhibits CSCs, raising the question if HectH9 alters CSCs by blocking the glucose metabolic flux. Intriguingly, administration of BI8626 in the presence of 2-DG further reduced CSC formation, suggesting that HectH9 regulates CSCs not only through glycolytic flux but likely by limiting ROS production. Further studies are needed to understand how HectH9 regulates CSCs *in vivo*. Finally, we demonstrated that BI8626 effectively inhibits the survival of prostate cancer cells but not the normal prostate epithelial cells, highlighting the therapeutic value of HectH9-targeted therapies (Figure 1).

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

The work was supported by an NIH grant [R01 CA211912], an NYS grant [DOH01-C31845GG] and the Susan G. Komen Career Catalyst Grant [CCR17480331].

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