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EDITORIAL

A novel glucose sensor fuelling cancer growth

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Metabolic reprogramming is a well-established hallmark of cancer cells, enabling them to meet increased nutrient and energy demands for cell proliferation. Warburg effect is considered the first discovered metabolic alteration in cancer. It is characterized by the preferential conversion of glucose to lactate even when oxygen is available, therefore being also known as aerobic glycolysis. With his discovery in the 1920s, Otto Warburg laid the foundation for the field of tumour metabolism. Over the years, technological advances based on analytical chemistry (metabolomics) and mathematical modelling-integrated approaches, further extended Warburg's pioneering observations adding layers of complexity to our understanding of mechanisms and functions of glucose metabolism in cancer. Beyond acting as a pivotal energy source and anaplerotic carbon supply for the tricarboxylic acid cycle, glucose metabolism accomplishes additional functions in cells. Glucose-derived acetyl-CoA and lactate are employed, respectively, for acetylation and lactylation of histones, thereby affecting epigenetics and gene expression. Also, glucose might act as a signalling molecule directly binding to its protein sensors in order to coordinate oncogenic processes, independently on its catabolism. For instance, the methyltransferase NOP2/Sun RNA methyltransferase 2 (NSUN2) has been recently identified as a glucose sensor which is directly activated by glucose. Glucose-bound NSUN2 maintains m^5C RNA methylation and stability of three prime repair exonuclease 2 (TREX2), an exonuclease that degrades cytosolic DNA. Stabilized TREX2 restricts activation of cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway, finally supporting malignant transformation and resistance to immunotherapy [1]. Beyond cancer, glucose was found to drive epidermal differentiation by interacting with the RNA-binding protein DExD-box helicase 21 (DDX21). Accumulation of intracellular glucose levels during keratinocytes differentiation results in glucose binding to DDX21. Such interaction supports the formation of protein complexes containing RNA splicing factors, thereby enhancing the splicing of essential pro-differentiation genes [2]. Although these reports highlight glucose-binding ability of signalling proteins, whether glycolytic enzymes can also act as glucose sensors and take part in signalling networks to drive specific cell outputs remains largely unknown.

In the manuscript entitled "HKDC1 functions as a glucose sensor and promotes metabolic adaptation and cancer growth via interaction with PHB2" published in the present issue of *Cell Death and Differentiation*, Huang group bridged this gap of knowledge and identified hexokinase domain-containing 1 (HKDC1) as a novel glucose sensor that promotes tumour growth by sequestering the multirole prohibitin 2 (PHB2) protein, disabling its inhibitory effect on the oncogenic transcription factor Sp1 [3]. Hexokinase (HK)-mediated glucose phosphorylation is the rate-limiting step in glycolysis. Up to date, five HK isozymes (HKI-IV and HKDC1) have been identified in mammalian cells, each with different kinetics and substrate affinity [4]. HKDC1 is the most recently identified human HK and previous reports demonstrated that its expression correlates with poor overall survival in cancer patients [5]. Interestingly, HKDC1 was found to interact with STAT1 to enhance PD-L1 expression, thereby promoting immune evasion in hepatic cancer, indicating that HKDC1 might have additional functions beyond its canonical enzymatic activity [5]. As complementary observation of previous reports, Liu and colleagues here shows that *HKDC1* is overexpressed in lung cancer patients, correlating with poor survival, suggesting that lung tumours take benefit from such enzyme for disease progression.

Moving from such evidence, the authors found that glucose deprivation decreases the protein levels of HKDC1, but not those of other HKs, in lung cancer cell lines cultured in vitro. This phenomenon is reversible, since glucose replenishment reverses HKDC1 suppression, suggesting that HKDC1 could act as a glucose sensor, able to regulate its own protein levels in response to glucose availability.

Importantly, glucose prevents HKDC1 degradation in a proteasome-dependent manner, since pharmacological proteasome inhibition blocked HKDC1 degradation under glucose-deprived conditions. Using both structural modelling approaches and site-specific mutagenesis, the authors identified two amino acidic residues required for regulating HKDC1 stability. Lys620, located in proximity of the glucose binding site, is the residue undergoing ubiquitination and, therefore, responsible of HKDC1 degradation under glucose-deprived conditions. When glucose is available, the binding of the sugar to HKDC1 masks such lysine residue, impairing its ubiquitination and subsequent protein degradation *via* the proteasome. Ser896 is the amino acid residue localized within the glucose binding pocket responsible for maintaining the structural conformation of the enzyme active site. In response to Ser896 to alanine substitution a structural change occurs, preventing Lys620 ubiquitination, and stabilizing HKDC1 even in the absence of glucose.

The authors demonstrated that HKDC1 deletion leads to a significant decrease in the glycolytic rate, balanced by a concomitant increase in beta oxidation, enabling cells to maintain stable ATP levels. Such evidence suggests that HKDC1 degradation might allow cancer cells to adapt metabolically to low glucose availability in the tumour microenvironment, by increasing the utilization of fatty acid as an alternative carbon source. In line with such findings, HKDC1-deficient human lung cancer cells are found more sensitive to etomoxir, a chemical inhibitor of fatty acyl chain import from the cytosol into the mitochondria for degradation. This suggests that increased fatty acid oxidation might be a vulnerable adaptation to HKDC1 deletion, possibly exploitable for clinical benefit. Etomoxir utilization for therapeutic purposes has been discontinued and its clinical development terminated due to severe side effects [6, 7]. Therefore, the repurposing of different fatty acid oxidation inhibitors already used in the clinics to treat cardiovascular diseases, such as Trimetazidine and Ranolazine, as anti-cancer agents in combination with glucose uptake inhibitors, might be envisioned.

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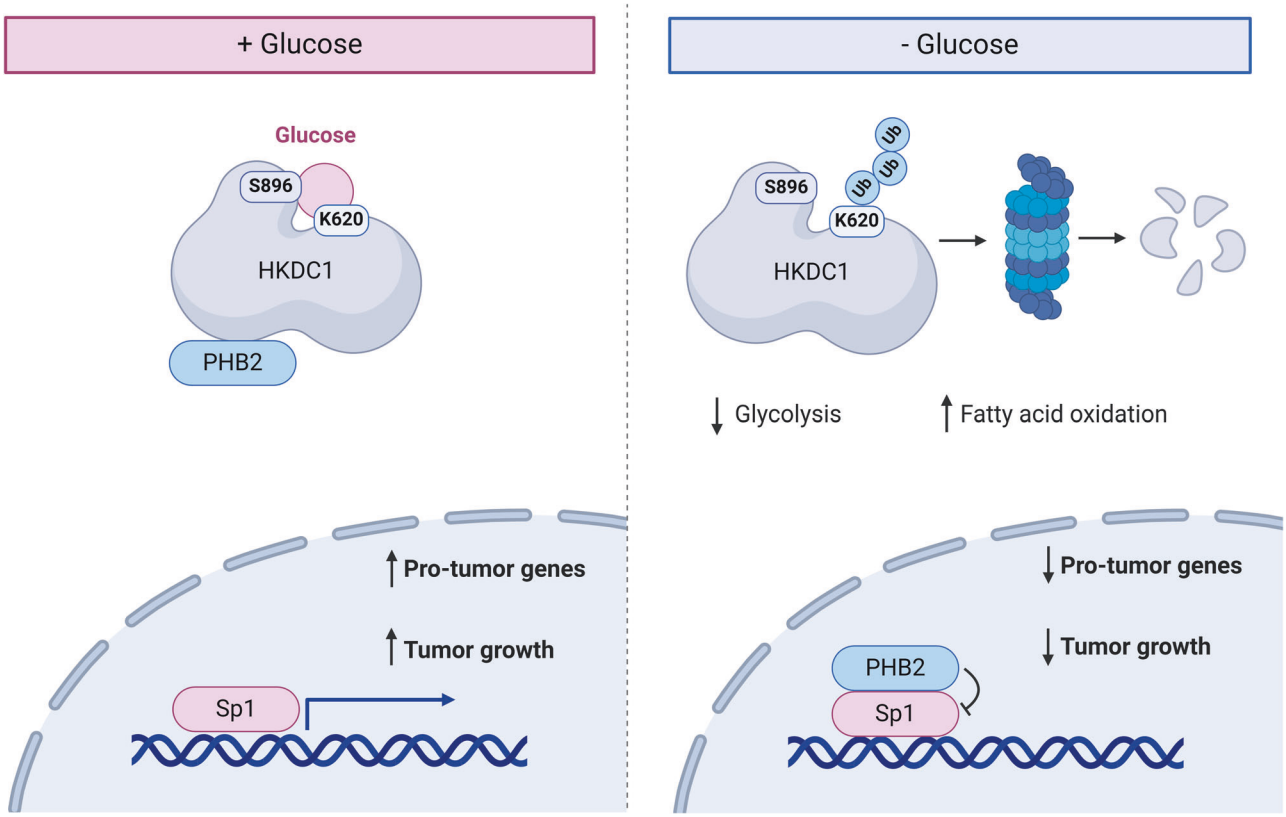

Fig. 1 Glucose-mediated stabilization of HKDC1 orchestrates an oncogenic programme in cancer cells. When glucose is available, it binds HKDC1 and masks Lys620, which hinders its ubiquitination and subsequent enzyme degradation by the proteasome. This stabilization allows HKDC1 to interact with and sequester PHB2, thereby impairing the PHB2's inhibitory effect on the transcription factor Sp1. As a consequence, Sp1 is able to promote the expression of pro-tumour genes, enabling cancer cells proliferation. In contrast, glucose depletion promotes the exposure of Lys620 for ubiquitination and subsequent proteasome-mediated degradation of HKDC1. This results in decreased glycolysis and a concomitant increased in fatty acid oxidation. Ser896 maintains the structural conformation of the glucose binding site, which allows accessibility and ubiquitination of Lys620. As a consequence of HKDC1 degradation, PHB2 is free to translocate to the nucleus, where it inhibits Sp1-mediated expression of pro-tumour genes, ultimately slowing tumour growth. Figure created with BioRender.

The authors also demonstrated that human HKDC1-ablated lung cancer cells display a decreased proliferation rate in vitro and reduced ability to form tumours when implanted in immunocompromised mice. Mechanistically, the tumour-supporting properties of HKDC1 depend on its capability to bind and inactivate PHB2. More in detail, when cancer cells are cultured in glucose-containing media, PHB2 is mainly located outside the nucleus and physically interacts with HKDC1. Such interaction prevents PHB2 from binding and inhibiting the transcription factor Sp1, which promotes the expression of several pro-tumour genes associated with stemness. In contrast, during glucose deprivation, HKDC1 degradation releases PHB2, enabling its translocation to the nucleus and interaction with Sp1, thereby suppressing its transcriptional activity and cancer cell proliferation (Fig. 1).

The overall findings reported in this study have significant implications in onco-immunometabolism. Firstly, the tumour microenvironment (TME) is characterized by poor vascularisation and consequent scarce nutrient availability [8]. Therefore, it would be interesting to investigate the extent to which glucose supply within the tumours affects HKDC1 stability in cancer cells. Beyond neoplastic cells, other cell types within the TME, such as anti-tumour T-cells are known to heavily rely on glucose metabolism for their effector functions, hence competing with cancer cells for its utilization [8]. At the same time, tumour supportive lymphocytes, such as regulatory T cells, are less reliant on glycolysis and avoid competition with cancer cells for glucose within the tumour, by utilizing alternative carbon sources [9, 10]. Therefore, future investigations might be envisioned to understand how changes in cell representation and function within the tumour stroma might indirectly affect HKDC1 stabilization in cancer cells, by regulating glucose availability in the TME.

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AUTHOR CONTRIBUTIONS

LR wrote the manuscript. SC revised the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.