

Reprogrammed Cellular Metabolism and O-GlcNAc Modification in Cancer

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The capability to reprogram cellular metabolism in order to most effectively support proliferating cancer cells has been emerging as a hallmark of cancer. As a result, there has been a resurgence of interest in the field of tumour metabolism. Additionally, this reappearance in interest may partly be attributed to the tremendous capability of the recent technological advancement to explore the relationship between cellular metabolism and cancer to an extent which was not possible before. The most fundamental trait of cancer cells involves their ability to sustain cell proliferation in an uncontrolled manner. Uncontrolled cell growth also require intracellular metabolic adjustment to meet the continual demand of energy and macromolecules by the proliferating cells [1]. This necessity is well known to be served by increased glucose uptake and anaerobic glycolysis by cancer cells, also known as the Warburg effect [2]. This shift in tumour metabolism is critical for supporting cancer cells as increased glycolysis allows the diversion of glycolytic intermediates into various biosynthetic pathways, including those generating nucleosides and amino acids. This, in turn, facilitates the biosynthesis of the macromolecules and organelles required for assembling new cells [3]. Moreover, the Warburg-like metabolism seems to be present in many rapidly dividing embryonic tissues, once again suggesting a role in supporting the large-scale biosynthetic programs that are required for active cell proliferation [1,4]. Biochemical and molecular studies suggest several possible mechanisms by which this metabolic alteration may evolve during cancer development. These mechanisms include mitochondrial defects and malfunction, adaptation to hypoxic tumour microenvironment, oncogenic signaling, and an abnormal expression of metabolic enzymes [5]. However, it is not known that such shift in metabolism also payback to cancer promoting proteins in a way which further facilitates their function or prevents their downregulation, thereby constituting a vicious circle in favour of cancer cells. One such potential mechanism may involve the hexosamine biosynthetic pathway (HBP) and the post-translational modification of protein by β -*N*-acetylglucosamine (*O*-GlcNAc); as changes in glucose uptake and metabolism also alter nutrient signaling pathways, including HBP [6,7]. The final product of HBP is uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc) [8]. The UDP-GlcNAc is a donor substrate for the post-translational modification at serine and threonine residues of a wide range of proteins including proteins known to be involved in the pathogenesis and progression of cancer (e.g. proteins enhancing glucose uptake, tumour suppressors, oncogenes, metabolic enzymes and mitochondrial proteins) [9]. For example, the constitutive activation of phosphatidylinositol 3 kinase/protein kinase B (PI3K/Akt) pathway is known to upregulates glucose uptake in cancer cells, and various components involved in this process are known to undergo *O*-GlcNAc modification [9,10]. Therefore, a potential exists for a positive feedback role of *O*-GlcNAc modification in facilitating reprogrammed metabolism in cancer cells. Currently our knowledge of *O*-GlcNAc modification of proteins and their role in the maintenance of cancer cell phenotype is very limited. This lack of knowledge may be due to the insufficient tools and techniques in the past for the identification and quantification of *O*-GlcNAc modification in proteins. With recent development in mass spectrometry and other associated technology it appears to be getting feasible to perform such analysis [11,12].

The *O*-GlcNAc modification is catalysed by the enzyme *O*-linked *N*-acetylglucosamine transferase (OGT), which transfers

N-acetylglucosamine from UDP-GlcNAc to protein substrates; whereas the enzyme *N*-acetyl- β -glucosaminidase (OGA), removes the *O*-GlcNAc modification from the modified protein [13]. Together, OGT and OGA dynamically alter the post-translational state and function of proteins in response to cellular signals [9]. *O*-GlcNAc modification is involved in extensive crosstalk with other post-translational modifications, such as phosphorylation including tyrosine phosphorylation and virtually all *O*-GlcNAc modified proteins are phosphoproteins [9,14,15]. As many of the processes that are perturbed in the pathogenesis and progression of cancer involved altered protein phosphorylation, changes in *O*-GlcNAc modification are likely to have effect on them. Such effects may also be mediated by altering the crosstalk between the multiple pathways diverging from growth factor receptors or by disruption of self-attenuating negative feedback thus facilitating their constitutive activation.

The redirection of energy metabolism in cancer cell is largely orchestrated by proteins that are involved in one way or another in programming the core hallmarks of cancer [1]. However, their interconnection with tumour metabolism and eventual integration into the hallmarks of cancer remain elusive. *O*-GlcNAc modification may allow cells to couple reprogrammed cell metabolism to factors/pathways known to support various hallmarks of cancer. For example, glycolytic fuelling has been shown to be associated with activated oncogenes (e.g., *RAS*, *MYC*) and mutant tumour suppressors (e.g., *TP53*) [16,17]. Moreover, hypoxic conditions that operates within many tumours, the hypoxia response system acts pleiotropically to upregulate glucose transporters and multiple enzymes of the glycolytic pathway [16-18]. Thus, both the Ras oncoprotein and hypoxia can independently increase the levels of the hypoxia-inducible factor (HIF) 1 α and HIF2 α transcription factors, which in turn upregulate glycolysis [18-20]. Interestingly a number of these proteins have been identified as *O*-GlcNAc modified proteins [21]. It is possible that *O*-GlcNAc modification alter their function in a way which further supports cancer cells. Furthermore, tumour suppressor protein Rb and P⁵³ operate as integration nodes for larger network that govern the decisions of cells to proliferate or alternatively activate senescence and apoptotic programs [1]. Tumour cells evolve a variety of strategies to limit or circumvent apoptosis, most commonly through the loss of P⁵³ tumour suppressor function which is known to be linked with increased glycolysis [22,23]. The stability of P⁵³ in cell is regulated by phosphorylation at multiple residues. Phosphorylation at Ser18 and Ser23 promotes P⁵³ stability whereas phosphorylation at Thr¹⁵⁵ promotes P⁵³ degradation [24]. The P⁵³ has been identified as an *O*-GlcNAc modified protein.

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Furthermore, it has been shown that O-GlcNAc modification of P⁵³ at Ser149 inversely correlates with Thr¹⁵⁵ phosphorylation [25]. Thus, altered O-GlcNAc modification of P⁵³ may contribute to the stability and function in cancer cells including its role in glycolysis. Similar reciprocal relationship between site specific phosphorylation and O-GlcNAc modification has been identified in the case of oncoprotein Myc [26,27]. Furthermore, oncogenes such as MYC, NF- κ B, AKT, tyrosine kinases and tumour suppressor genes including TP53 and PTEN that have been linked to increased glycolysis, have also been implicated in the upregulation of glutamine [28]. Glutamine function as a source of both nitrogen and carbon and contains amino and amido nitrogens, which are transferred to metabolic intermediates in the synthesis of nucleic acids, proteins, and hexosamines making it a crucial nutrient during cell proliferation [29,30]. It is not surprising that novel imaging strategies focusing on glutamine that could provide a valuable complement to ¹⁸F-FDG PET, because glutamine complements glucose in the metabolic platforms that support tumour growth at the cellular level [29]. It should be noted that hexosamine biosynthesis integrates glucose and glutamine metabolism because the rate limiting step is the addition of the α -amido group of glutamine to a hexose sugar by the enzyme glutamine fructose-6-phosphate amidotransferase [8]. Thus, tumour metabolism provides a favourable condition for the upregulation of O-GlcNAc levels in cancer and high GlcNAc levels have been shown to play a role in glutamine-dependent cell growth and proliferation [31]. Abnormal levels of O-GlcNAc in cancer cells may payback by altering post-translational control of protein function linked to oncogenic phenotypes.

A developmental regulatory program, referred to as the epithelial-mesenchymal transition (EMT), has become prominently implicated as a means by which transformed epithelial cells can acquire the abilities to invade, to resist apoptosis, and to disseminate [32-36]. This multifaceted EMT program can be activated transiently or stably, and to differing degrees, by carcinoma cells during the course of invasion and metastasis. An upregulation of O-GlcNAc levels have been implicated in EMT through the regulation of phosphorylation and ubiquitination of transcription repressor SNAIL1 that target E-cadherin [37]. Moreover, as UDP-GlcNAc, the product of HBP is not only required for OGT mediated O-GlcNAc modification of cytosolic and nuclear proteins but also in the glycosylation of membrane proteins, it is possible that high GlcNAc levels may contribute in EMT by changing the topology of cell surface proteins. Alternatively, O-GlcNAc effect could be mediated through altered O-GlcNAc modification of transcription factors that have been implicated in EMT [38].

Given an emerging role of O-GlcNAc modification as a fundamental regulatory mechanism involved in the various cellular processes it is highly unlikely that global approaches such as RNAi mediated knockdown of OGT and OGA or their overexpression, inhibiting OGT and OGA activity by small molecule inhibitors would provide a definite answer regarding protein specific role of O-GlcNAc modification in cancer cell metabolism or cancer phenotype. It would be very challenging to distinguish the target specific effect from non-target effect of such global approaches. Although it has been proposed that increased glucose uptake/glycolysis in cancer cells may lead to increased glucose flux through HBP and subsequently increased O-GlcNAc modification in a number of proteins [7,30]. However, to the best of my knowledge no attempt has been made to study the effect of inhibitors of glycolysis on HBP, O-GlcNAc level and O-GlcNAc protein modification in cancer cells. Furthermore, such hypothesis is not tenable to explain the simultaneous downregulation of O-GlcNAc modification in a number of proteins in cancer cells [30]. Although not

well understood, such findings are not surprising, keeping in mind that O-GlcNAc modification in protein is a highly regulated process and involved not only O-GlcNAc level, O-GlcNAc cycling enzymes OGT and OGA but also their interacting proteins and various modification status of the substrate itself [9,15]. Delineating the role of O-GlcNAc modification in relation cancer cell metabolism and function would require not only protein specific but also modification site specific approaches, as proteins are often subjected to a number of post-translational modifications, often with very different and even opposite functional consequences. A better understanding of the underlying mechanisms could provide opportunities for the development of i) novel therapeutic agents to disrupt the vicious cycle of metabolism and cell signaling pathways which is critical for cancer phenotype, and ii) a rationale for simultaneous targeting of reprogrammed metabolism and cell signaling pathways.

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