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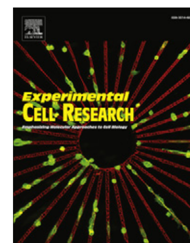

Research Article

Keratin 8/18 regulation of glucose metabolism in normal versus cancerous hepatic cells through differential modulation of hexokinase status and insulin signaling

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ABSTRACT

As differentiated cells, hepatocytes primarily metabolize glucose for ATP production through oxidative phosphorylation of glycolytic pyruvate, whereas proliferative hepatocellular carcinoma (HCC) cells undergo a metabolic shift to aerobic glycolysis despite oxygen availability. Keratins, the intermediate filament (IF) proteins of epithelial cells, are expressed as pairs in a lineage/differentiation manner. Hepatocyte and HCC (hepatoma) cell IFs are made solely of keratins 8/18 (K8/K18), thus providing models of choice to address K8/K18 IF functions in normal and cancerous epithelial cells. Here, we demonstrate distinctive increases in glucose uptake, glucose-6-phosphate formation, lactate release, and glycogen formation in K8/K18 IF-lacking hepatocytes and/or hepatoma cells versus their respective IF-containing counterparts. We also show that the K8/K18-dependent glucose uptake/G6P formation is linked to alterations in hexokinase I/II/IV content and localization at mitochondria, with little effect on GLUT1 status. In addition, we find that the insulin-stimulated glycogen formation in normal hepatocytes involves the main PI-3 kinase-dependent signaling pathway and that the K8/K18 IF loss makes them more efficient glycogen producers. In comparison, the higher insulin-dependent glycogen formation in K8/K18 IF-lacking hepatoma cells is associated with a signaling occurring through a mTOR-dependent pathway, along with an augmentation in cell proliferative activity. Together, the results uncover a key K8/K18 regulation of glucose metabolism in normal and cancerous hepatic cells through differential modulations of mitochondrial HK status and insulin-mediated signaling.

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Abbreviations: HCC, hepatocellular carcinoma; K, keratin; IF, intermediate filament; G6P, glucose-6-phosphate; HK, hexokinase; GK, glucokinase; VDAC, voltage-dependent anion channel; GS, glycogen synthase; PI3K, phosphatidylinositol-3 kinase; GSK-3β, glycogen synthase kinase-3β; mTOR, mammalian target of rapamycin; K8-null, K8-knockout hepatocytes; WT, wild-type hepatocytes; shK8b, K8-knockdown hepatoma cell line; H4ev, wild-type hepatoma cell line; Wort, wortmannin; Rapa, rapamycin

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Introduction

Glucose is of fundamental importance to cells, where distinct regulatory mechanisms have evolved to control its uptake and utilization in differentiated versus proliferating cells [1,2]. Differentiated cells, such as hepatocytes, primarily utilize glucose for ATP production through oxidative phosphorylation of glycolysis-derived pyruvate, whereas proliferating cancer cells, such as those from hepatocellular carcinoma (HCC), undergo a metabolic shift to aerobic glycolysis, irrespective of oxygen supply, a phenomenon referred to as the Warburg effect [3]. While a conclusive explanation for this metabolic shift has remained elusive over the years, recent work has provided enlightening evidence, by which the metabolism of rapidly dividing cells, including normal embryonic cells, is adapted to facilitate the utilization of glucose as a carbon source for the production of constituents required to generate a new cell [2]. According to this scheme, normal cell proliferation is dependent on the interplay between growth-related signaling and glycolytic pathways, and in cancer cells this dependence is overcome by the acquisition of genetic mutations that functionally affect receptor-initiated signaling. The work reported here addresses the cytoskeletal keratin involvement in the interplay between glucose utilization and insulin signaling in normal versus cancerous hepatic cells.

Glucose uptake requires GLUTs, members of the SLC2A family of membrane transport proteins, which exhibit wide variations in tissue distribution [4,5]. Once inside the cell, its utilization is initiated following its conversion to glucose-6-phosphate (G6P) by a hexokinase (HK), a phosphorylation event that locks its entry and initiates its metabolism, mainly through glycolysis [1,6]. While HK constitutes a family of 4 isoforms (I–IV) largely expressed in a cell type-dependent fashion in normal adult tissues, their expression is often restricted to HKI and HKII in malignant tumors [1]. Normal hepatocytes express all four isoforms at different levels, with HKIV (referred to as glucokinase (GK)), being prominent at the adult stage [4,7], whereas human hepatocellular carcinoma (HCC) and rat hepatoma cells predominantly express HKII alone or HKI/HKII [1,7]. Mechanistically, there is evidence indicating that GK activation is regulated through an actin-dependent nuclear/cytoplasmic process [8,9], whereas HKII is targeted at the mitochondrial membrane, where it binds to voltage-dependent anion channel (VDAC) [10], at domains that also associate with actin-binding proteins and free tubulin [11], thus suggesting that HK activation and intracellular localization implicate a cytoskeleton contribution.

Although a deregulation between glucose uptake and production in hepatocytes largely contributes to the onset of insulin-related chronic diseases [12], there is accumulating evidence supporting metabolic connections between diabetic conditions and various malignancies, and also between glycogen storage disease and HCC [13,14]. Normally, elevated blood glucose triggers the release of insulin by pancreatic β-cells, and in turn insulin modulates glycogen formation in hepatocytes through a regulation of glycogen synthase (GS) [15]. Accordingly, insulin binding to its cognate receptor at the surface of target cells leads to activation of phosphatidylinositol-3 kinase (PI3K) [16], along with pleckstrin homology domain-containing kinases PDK-1 and Akt. In turn, activated Akt phosphorylates multiple downstream effectors, including HKII in link with its mitochondrial translocation [17]. In addition, Akt inhibits by phosphorylation the activity of glycogen synthase kinase-3β (GSK-3β), and since GSK-3β inhibits GS also by phosphorylation, suppression of GSK-3β leads to activation of glycogen synthesis [18]. However, another substrate of interest for Akt is the mammalian target of rapamycin (mTOR), within a context of protein synthesis and cell growth [19,20]. Thus, insulin can activate signaling pathways that regulate not only glycogen formation, but also protein synthesis and cell growth.

Keratins (Ks), the intermediate filament (IF) proteins of epithelial cells, constitute the largest family of cytoskeletal proteins, which are grouped into type I (K9–28) and type II (K1–K8 and K71–K80) subfamilies [21]. Keratin IFs are obligate heteropolymers that include at least one type I keratin and one type II keratin, and are coordinately expressed as specific pairs in a cell lineage and differentiation manner. Notably, cells of simple epithelia express 4–6 keratins as different partners, with the K8/K18 pair being common to all of them [22,23]. In addition, epithelial cancers largely maintain the keratin expression associated with their respective cell type of origin, which means that keratins have long been recognized as diagnostic markers of tumor pathology [24–26]. Moreover, in line with the emergence of keratins as multifunctional regulators of epithelial malignancies [24], the persistence of K8/K18 IFs is a hallmark of invasive squamous cell carcinoma, where such perturbed K8/K18 expression appears to contribute to cell invasiveness [26,27]. Of particular note, hepatocyte and hepatoma cell IFs consist of the K8/K18 pair only [22,25], and point mutations in their genes in hepatocytes lead to IF disorganization and predispose to liver cirrhosis [23], a tissue alteration often associated with the emergence of HCC [28]. In addition, we have reported recently that K8/K18 IFs modulate the substratum-dependent adhesion and migration of cultured hepatocytes and hepatoma cells in a differential manner [29,30], and also the FasR-induced apoptosis and reactive oxygen species (ROS)-mediated death [31–34]. In the study reported here, we used cultured K8-knockout (K8-null) and wild-type (WT) hepatocytes versus K8-knockdown (shK8b) and wild-type (H4ev) hepatoma cell clones to investigate the K8/K18 IF involvement in the regulation of glucose uptake and phosphorylation, GLUT and HK status, lactate release and insulin-stimulated glycogen formation. The results uncover a distinctive K8/K18 IF regulation of glucose utilization in hepatocytes versus hepatoma cells, through a differential modulation of the glucose-metabolic and insulin-signaling pathway interplay.

Materials and methods

Reagents

Engelbreth-Holm-Swarm (EHS) Matrigel (#354234) was purchased from BD Pharmingen (Mississauga, ON, Canada). Anthrone (#A1631), insulin (#I6634), LiCl (#L9650), rapamycin (#R0395), wortmannin (#W1628) and all other chemicals were from Sigma-Aldrich Canada Ltd (Oakville, ON, Canada). *Primary antibodies used were*: Rabbit polyclonal anti-GLUT-2 (#07-1402), anti-Hexokinase type I (#AB3543) and anti-Glycogen Synthase (CT; #04-357) from Millipore (Temacula, CA, USA); rabbit polyclonal anti-Insulin Receptor β (C19; #sc-711) and anti-GK (GCK; sc-7908) from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA,

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