SIRT6, FOXO4, HIF3A: UNLOCKING KEY METABOLIC REGULATORS IN CANCER CELLS

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ABSTRACT

In recent years, there has been an expanding interest in understanding metabolic regulation in cancer cells. Since cancer cells are characterized by accelerated rate of proliferation, their metabolic demands are increased. The latter forces cancer cells to facilitate metabolic pathways, which are not normally active in healthy cells. In order to achieve this, cancer cells use the regulatory machinery of these pathways to survive and acquire resistance against defense mechanisms of molecular or cellular origin. In this review, we focus on three regulatory genes SIRT6, FOXO4, HIF3A, which orchestrate basic metabolic pathways, such as energy metabolism, and regulate proliferation. Their expression is altered in conjunction with cancer type and stage. In this way, they can act as oncogenes or tumor suppressors. *Scr Sci Med.* 2017;49(4):22-28

Keywords: cancer metabolism, SIRT6, FOXO4, HIF3A

INTRODUCTION

Metabolic reprogramming is one of the key hallmarks of cancer progression. Many efforts have been made to understand key factors that orchestrate switches in metabolic pathways, which in turn will provide the necessary macromolecules to answer cancer cell demands (1). It has been extensively mentioned that sirtuins, FOXO transcription factors as well as hypoxia-inducible factors possess dominant functions towards organizing cell metabolism

under different conditions (2-4). In the current review, we propose a possible interaction mechanism between SIRT6, FOXO4 and HIF3A by combining already known interactions and signal transduction pathways in order to elucidate possible implication of these genes in cancer metabolic reprogramming. Additionally, we summarize important findings relating the function of these genes to the cancer type and stage.

Metabolic reprogramming of cancer cells

During the past decade, substantial efforts have been made towards understanding the mechanisms and biologic effects associated with metabolic reprogramming in cancer. Metabolic reprogramming together with mutations in oncogenes and tumor suppressors lead to alterations in gene expression as well as epigenetic changes and post translational modifications do (1). One characteristic and well-known reprogrammed metabolic pathway in cancer is the Warburg effect or aerobic glycolysis (5). Under aerobic conditions, normal cells utilize glucose to pro-

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duce pyruvate through glycolysis and then pyruvate supplies adenosine triphosphate (ATP) through respiration in mitochondria by consuming oxygen. On the other hand, under anaerobic conditions, less pyruvate is produced and transported to mitochondria, thus favoring anaerobic glycolysis (6). Otto Warburg, about a century ago, observed that cancer cells constitutively take up glucose and produce lactate regardless of oxygen availability, and bypass the basic metabolic source of energy through respiration (7). In order to fulfill their increased demands on glucose, cancer cells upregulate GLUT1 transporters so as to transport high amounts of glucose in the cytoplasm (8). The increase in glycolytic flux allows diversion of glycolytic intermediates to supply subsidiary pathways to answer the monomeric substrate demands of proliferating cells, such as nucleosides and amino acids (5,9). However, tricarboxylic acid (TCA) cycle intermediates are also used as substrates for macromolecule synthesis and specifically the amino acid pool necessary for cancer proliferation (10). The pathways that replace the utilized and thus lost metabolites are called anaplerotic pathways, and they generate TCA cycle intermediates that can enter the cycle (11). Two important processes that provide anaplerotic fluxes in cancer cells are glutaminolysis, which produces α -ketoglutarate from glutamine, and pyruvate carboxylation, which produces oxaloacetate from glucose/pyruvate (1). Cancer cells comprising a tumor consist of two metabolic groups: i) the glucose dependent ones, which proliferate under hypoxic conditions and release lactate as a waste product and ii) the lactate utilizing tumor cells, which are situated in areas of more abundant vessels and thus better oxygenated (6). Taken into consideration the unorganized vasculature and shunts creating this regional hypoxia, tumor cells develop symbiotic metabolic behavior to utilize the lactate produced through aerobic glycolysis (Warburg effect) and anaerobic glycolysis (Pasteur effect) (12). Moreover, oncogenes, such as RAS, and mutations on suppressors, such as p53 promote cancer cell proliferation and anti-apoptotic mechanisms by influencing on the glycolytic pathway (13). The hypoxic conditions established within tumors, together, but independently, with Ras oncoprotein, increase the levels of the HIF1A and HIF2A transcription factors, favoring glycolysis (4,14). As a result, energy metabolic reprogramming can be con-

sidered a hallmark of cancer, since oncogenes, tumor suppressors and last but not least, transcription factors orchestrate metabolic regulation of fast growing and proliferating cancer cells (6).

The impact of sirtuins on cancer

It is widely known that sirtuins affect extensively processes of cancer cells. They can act as tumor suppressor and/or oncogene, depending on various genetic contexts, tumor types and stages (15). SIRT1 is up-regulated in malignant cells or tissues from patients with leukemia, glioblastoma, prostate, colorectal, or skin cancer through its anti-apoptotic activities by repressing p53 and FOXO transcription factors (16). On the other hand, in breast cancer, SIRT1 is down-regulated in association with BRCA1 mutations and acts as tumor suppressor, as it controls positively the expression of the anti-apoptotic surviving (17). SIRT2 is associated with cancer metabolism by affecting the response against hypoxia, since it regulates HIF1 α expression and thus aerobic glycolysis (18). Depending on different types of cancer and stage, SIRT2 is up-regulated or down-regulated (15). SIRT3 acts primarily as a tumor suppressor by repressing HIF1A and thus inhibiting the switch to aerobic glycolysis (19). Low SIRT4 expression levels are associated with several types of cancer including lung tumors, where patients have poorer survival rate (20). SIRT5 affects glucose metabolism by suppressing pyruvate dehydrogenase (PDH) and succinate dehydrogenase (SDH) leading to hypoxia response and tumor-promoting stimulus (21).

The dual role of SIRT6

Recently, there is an emerging interest in understanding SIRT6 regulatory effects and biologic roles. SIRT6 functions as an ADP-ribosylase and NAD⁺-dependent deacetylase of both acetyl groups and long-chain fatty acyl groups and regulates DNA repair, telomere maintenance and glucose and lipid metabolism, thus affecting metabolic and age related diseases such as cancer (2). As mentioned before, enhanced glycolysis under aerobic conditions is a characteristic process of metabolic reprogramming in cancer cells (22). It has been shown that the loss of SIRT6 de-represses HIF1A-dependent transactivation of glycolytic genes and leads to enhanced glucose uptake and increased glycolysis with concomitant inhibition of mitochondrial respiration (23).

Upon experiments on mouse embryonic fibroblasts (MEFs) loss of SIRT6 lead to oncogene activation and tumor formation, which exhibits enhanced aerobic glycolysis. Additionally, SIRT6 suppresses aerobic glycolysis through co-repressing HIF1A and co-represses MYC transcriptional activity of ribosomal genes (24). According to Kugel and Mostoslavsky (2014), SIRT6 expression is downregulated in human pancreatic ductal carcinoma and colorectal carcinomas compared to normal samples, and concomitantly, the expression of the HIF1 α -target genes GLUT1, LDH, and PFK1 is significantly upregulated in these samples. Furthermore, low SIRT6 levels negatively correlate to cancer progression and/or survival in colorectal cancer (2). Moreover, SIRT6 acts as a tumor suppressor, inhibiting the initiation and progression of colorectal cancer *in vivo* by repressing HIF1A and MYC, thereby inhibiting aerobic glycolysis and ribosomal biogenesis gene expression, respectively (Fig. 1) (24). Other studies show that in early stage liver cell carcinoma, SIRT6 inhibits anti-apoptotic activity through NF- κ B activation (25). Similarly, SIRT6 is downregulated at early stages of head and neck cancers (26). On the other hand, in squamous cell carcinomas, SIRT6 is upregulated, as the negative effector microRNA-34a is downregulated, and thus SIRT6 expression levels are increased (26). SIRT6 knockdown stimulates the increased miR34a levels on differentiation in human keratinocytes and squamous cell carcinomas (26). Patients with chronic lymphocytic leukemia have increased SIRT6 expression levels compared to healthy individuals and these higher levels are associated with poorer prognosis in such patients (27). These results show the dual role of SIRT6. The effects of SIRT6 on metabolism can be secondary to overexpression of SIRT6 to provide genomic stability. Probably at early cancer stages, low expression causes genomic instability and metabolic reprogramming leading to accumulation of mutations and swift enhancement towards metabolic pathways and metabolites necessary for cancer cell proliferation (2). Additionally, the accelerated proliferation together with genomic instability could allow subsequent mutations in oncogenes, such as KRAS. At advanced stages, high SIRT6 activity may protect against accumulation of further mutations, affecting negatively tumor growth (2).

Figure 1. A graph of possible interaction pathways between SIRT6, FOXO4 and HIF3A

FOXO transcription factors in cancer

FOXO transcription factors play an important role in cell functions. These functions are regulated at post-transcriptional level modification mainly through phosphorylation by kinase signaling pathways including Akt, SGK, ERK and IKK, which can be dysregulated in cancer suggesting the implication of FOXO factors in tumor evolution processes (28,29). Important FOXO transcription factors are FOXO1, FOXO3, FOXO4 and FOXO6, with the first three expressed in all types of tissue with tissue-dependent expression profile, while FOXO6 is expressed exclusively in the central nervous system (30,31). Under oxidative and nutrient stress conditions, FOXOs become phosphorylated and translocated from the cytoplasm to the nucleus to induce the expression of genes involved in energy metabolism and stress resistance (32). On stimulation of PI3K AKT signaling by growth factors through binding on JAK receptors, AKT phosphorylates FOXOs, which leads to their cytoplasmic sequestration and inactivation (Fig. 1) (33,34). FOXO factors show controversial functions since they possess anti-proliferative and pro-apop-

totic functions, together with cancer drug resistance, maintenance of leukemia-initiating cells and colon cancer metastasis (3).

FOXO4

FOXO4 functions as tumor suppressor by inhibiting cell proliferation, inducing apoptosis and protecting cells from DNA damage and oxidative stress (35,36). Under epidermal growth factor positive influence, FOXO4 is phosphorylated leading to downregulation of ANXA8 expression, which is responsible for epithelial-to-mesenchymal transformation (37). Thus FOXO4 plays key roles in growth factor-mediated tumor metastasis during the epithelial-to-mesenchymal transition change in cholangiocarcinoma (37,38). Furthermore, it has been demonstrated that FOXO4 is regulated by posttranslational modification through miR-499-5p overexpression, which binds to the 3'-UTR of FOXO4, dramatically decreasing the level of FOXO4 mRNA and protein expression in colorectal cancer cells (38). Additionally, knockdown of miR-499-5p by siRNA suppresses the migration capacity and invasiveness of colorectal cancer cells (38).

Hypoxia-inducible factors

Hypoxia-inducible factors (HIFs) are transcription factors activated in response to hypoxia and activate different signaling pathways. These pathways in turn alter cellular responses including those of cancer cells (39). Highly proliferative cancer cells overconsume oxygen carried by the bloodstream, thus creating intratumoral areas with hypoxic environment (40). In order to survive, cancer cells have to adapt to these conditions to ensure their high proliferative rate (41). Gene expression is suppressed to conserve energy for the essential metabolic functions (42). HIF1 and HIF2 are important regulators of the cellular response to hypoxia, altering gene expression to promote angiogenesis, glucose metabolism and resistance to oxidative stress. Their key regulatory subunits, HIF1A (HIF-1 α) and endothelial PAS domain protein 1 (or HIF2A) are overexpressed and positively associated with disease progression in a variety of cancers (43). It has been shown that hypoxia induces expression of various genes including vascular endothelial growth factor (VEGF) to promote neoangiogenesis and several anti-apoptotic genes, such as KDM3A, PGK1, TGFB1, and SLC2A3 (GLUT3) (44). In-

terestingly, HIF1A expression in healthy stromal and cancer cells induces metabolic shift towards aerobic glycolysis with increased glucose uptake and lactate production and mitochondrial activity inhibition (45). In cancer cells solely, HIF1A acts as tumor suppressor. On the contrary, HIF2A induces genes like EGFR, Ras and cyclin D1, known to promote cancer proliferation, and, additionally, enhances mitochondrial oxidative activity. As a result, this differential expression and function between stromal cells and cancer ones creates a symbiotic system, where healthy stromal cells produce and provide paracrine lactate to the proliferating cancer cells (45). However, HIF1A overexpression is significantly associated with higher colorectal cancer-specific mortality rate in contrast to HIF2, the expression of which is not related to patient survival (43).

HIF3A

Unlike HIF1A and HIF2A, there is limited knowledge on HIF3A function and regulation. HIF3A produces a number of different mRNA transcripts mostly by alternative splicing (46). HIF3A is induced by hypoxia, but the effects on cell response are indirect, as HIF3A overexpression inhibits the transcriptional activation of hypoxia reporter by HIF-1 and HIF-2 (47). On the other hand, siRNA knock-down of the endogenous HIF-3A variants leads to downregulation of certain HIF target genes, while overexpression of individual long HIF-3A variants exerts the opposite effect, showing the versatility and complexity of the alternate variants (47). Another study shows that HIF-3A1 overexpression greatly increases colorectal tumor cell growth by activating the pro-survival STAT3 signaling through binding to the upstream JAK (Fig. 1) (39). Tissue specimen from human colorectal tumors show HIF3A overexpression and is related to poor prognosis (39).

Discussion

In the recent years, substantial effort has been made to understand cancer metabolic reprogramming. Many studies have been performed on genomic, translational and post-translational level in an effort to unlock the regulatory mechanism of the processes leading to altered metabolism. Sirtuins regulate multiple functions necessary for cellular homeostasis, such as energy metabolism, stress response, DNA repair and genomic stability. Among

other functions, SIRT6, especially, regulates the energy metabolism and, more specifically, the loss of SIRT6 enhances glycolysis through HIF-1A potentiation. HIFs promote angiogenesis and enhance glycolysis in cancer cells. Additionally, the overexpression of the related HIF3A increases colorectal cancer tumor growth by activating pro-survival STAT3 signaling. FOXO transcription factors form another important group of regulatory genes involved in energy metabolism and stress resistance. Stress conditions and growth factors stimulate the PI3K AKT signaling through activation of JAK receptors, leading to FOXO phosphorylation, which in turn results in their cytoplasmic sequestration and inactivation. Assuming that the same pathway inactivates FOXO4 transcription factor, we develop a possible interaction scheme to interpret the interconnections between SIRT6, FOXO4, and HIF3A in an attempt to target a main switch in metabolic reprogramming (Fig. 1).

Conclusion

In conclusion, a great interest has emerged to assess combined gene expression patterns of SIRT6, FOXO4, and HIF3A in colorectal cancer patients in order to further illuminate the regulation of cancer metabolic reprogramming.

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