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"Warburg Effect" and Mitochondrial Metabolism in Skin Cancer

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Abstract

Skin cancer is the most common type of cancer in the United States, with an increasing annual rate. Defining the mechanism of skin cancer malignancy and progression is the first step towards skin cancer prevention and therapy. "Warburg Effect" describes the preference of glycolysis and lactate fermentation rather than oxidative phosphorylation for energy production in cancer cells, which also present in non-melanoma and melanoma skin cancers. Mitochondrial metabolism is an important and necessary component in the functioning and maintenance of the organelle, and accumulating evidence suggests that dysfunction of mitochondrial metabolism plays a role in skin cancer. Recently, new progress has demonstrated the mechanisms of the mitochondrial metabolism-to-glycolysis switch in skin cancer development and how to target this metabolic switch for skin cancer prevention and therapy, which will be discussed in this review.

Keywords: Skin cancer; Melanoma; Glycolysis; Mitochondrial metabolism; Mitochondrial respiration

Introduction

In vertebrates, food is digested and supplied to cells mainly in the form of glucose. Glucose is broken down further to make Adenosine Triphosphate (ATP) by two pathways. One is via anaerobic metabolism occurring in the cytoplasm, also known as glycolysis. The major physiological significance of glycolysis lies in making ATP quickly, but in a minuscule amount. The breakdown process continues in the mitochondria via the Krebs's cycle coupled with oxidative phosphorylation, which is more efficient for ATP production. Cancer cells seem to be well-adjust to glycolysis. In the 1920s, Otto Warburg first proposed that cancer cells show increased levels of glucose consumption and lactate fermentation even in the presence of ample oxygen (known as "Warburg Effect") [1]. Based on this theory, oxidative phosphorylation switches to glycolysis which promotes the proliferation of cancer cells [2]. Many studies have demonstrated glycolysis as the main metabolic pathway in cancer cells [3-6].

Why cancer cells prefer glycolysis, an inefficient metabolic pathway? It is now accepted that glycolysis provides cancer cells with the most abundant extracellular nutrient, glucose, to make ample ATP metabolic intermediates, such as ribose sugars, glycerol and citrate, nonessential amino acids, and the oxidative pentose phosphate pathway, which serve as building blocks for cancer cells [7].

Since, cancer cells have increased rates of aerobic glycolysis, investigators argue over the function of mitochondria in cancer cells. Mitochondrion, a one of the smaller organelles, produces most of the energy in the form of ATP to supply the body. In Warburg's theory, the function of cellular mitochondrial respiration is dampened and mitochondria are not fully functional. There are many studies backing this theory. A recent review on hypoxia nicely summarizes some current studies and speculates that the "Warburg Effect" provides a benefit to the tumor not by increasing glycolysis but by decreasing mitochondrial activity [8]. However, there are also studies pointing to an opposing direction. For instance, the oncogene Myc has been shown to increase oxygen consumption and mitochondrial mass and function via transcriptional activation of a number of its target genes involved in various mitochondrial functions. These gene products include Transcription Factor A, Mitochondrial (TFAM) [9], acetyl-CoA acetyltransferase, isocitrate dehydrogenase, L38, B-cell receptor-Associated Protein (BAP) 37, Heat Shock Protein (HSP) 10, prohibitin, inner membrane translocase, etc; [10].

In this review, new progress on the "Warburg Effect" on skin cancer, the possible molecular mechanisms of this metabolic switch, and the approaches to target this metabolic switch for skin cancer prevention and therapy will be discussed.

Mitochondrial respiration and skin cancer

Cellular respiration converts energy from nutrients into ATP while utilizing oxygen, known as aerobic respiration, or in the absence of oxygen, otherwise known as anaerobic respiration. The respiration in mitochondria belongs to the former, and glycolysis belongs to the latter. Mitochondrion is an intracellular organelle critically involved in regulating biogenesis, apoptosis, and oxidative stress. During cancer development, these mitochondrial functions also undergo substantial changes to facilitate cancer cell growth. Recent literature provides controversial results regarding mitochondrial biogenesis in skin cancer, and there is no absolute answer to whether mitochondrial biogenesis is down- or up-regulated in skin cancer development. Our speculation to this controversy is that the changes in mitochondrial metabolism may be cancer type- and stage- dependent.

Simulated Sunlight Irradiation (SSI) is a major cause of human skin cancer. When human malignant amelanotic melanoma A375 cells are exposed to SSI, mitochondrial dynamics and rate of mitophagy are significantly increased, which can be inhibited by glutamine, suggesting

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that damage to mitochondrial dynamics and mitophagy could play an important role in skin cancer development [11].

In a skin cell transformation model (murine epidermal JB6 P+ cells) [12], and in mouse skin tissues, we have demonstrated that mitochondrial respiration is dampened upon tumor promoter treatment [13]. This phenomenon is inhibited when Manganese Superoxide Dismutase (MnSOD) is overexpressed. Interestingly, mitochondrial respiration substrates are able to block this metabolic switch and subsequent skin cell transformation.

However, the following studies on established skin cancer samples show opposite results.

Arsenic-induced Bowen's disease (As-BD, a cutaneous carcinoma in situ), which is able to transform into invasive Basal Cell Carcinoma (BCC) and Squamous Cell Carcinoma (SCC), is one of the major health issues of arsenic poisoning in certain developing countries [14]. In the As-BD patients, genes involved in mitochondrial biogenesis are upregulated. These genes include cytochrome *c* oxidase, peroxisome Proliferator-activated receptor Gamma Coactivator-1α (PGC-1α), Nuclear Respiratory Factor 1 (NRF-1), and mitochondrial Transcription Factor A (mtTFA). Furthermore, mitochondrial oxygen consumption and intracellular ATP levels are increased in arsenic-treated keratinocytes [15]. These results suggest that increased mitochondrial biogenesis may contribute to arsenic-induced skin cell proliferation.

Similar results have also been observed in mitochondrial gene depleted B16 mouse melanoma cells. These cells show delayed subcutaneous tumor growth and failed to form metastatic lung tumors [16].

What are the master regulators that cause changes in mitochondrial metabolism and biogenesis? Studies suggest that (mitochondrial) DNA damage and non-mitochondria-derived free radicals are on top of the list.

The Xeroderma Pigmentosum C (XPC) protein plays a key role in DNA repair, and patients with XPC deficiency show increased incidence of skin cancer [17]. In human keratinocytes, knockdown of XPC reduced mitochondrial oxidative phosphorylation whereas glycolysis was increased [18]. In addition, XPC knockdown induced deletions in mitochondrial DNA (mtDNA) and Reactive Oxygen Species (ROS) production via NADPH oxidase (Nox) 1 (Nox1).

Akt, a serine/threonine protein kinase, is involved in multiple cellular processes such as DNA damage/repair, glucose metabolism, cell proliferation, apoptosis, transcription and cell migration. Studies have confirmed that Akt is also relevant to melanoma malignancy. In WM35 melanoma cells, overexpression of Akt increased the levels of ROS, and stabilized cells with mitochondrial DNA mutations, which can generate ROS. Increased ROS production may be mediated by induced Nox4 [19]. It has been confirmed that Nox1 contributes to the oncogenic ras transformation phenotype [20] and Nox4 promotes cell survival of cancer cells [21]. Maki Yamaura and collegues found that Nox4 was overexpressed in melanoma. Nox4-generated ROS are functionally required for melanoma cell transformation and advanced melanoma growth [22].

Glycolysis and skin cancer

Unlike the controversy on the role of mitochondrial metabolism and biogenesis during cancer development, it is well accepted that glycolysis is enhanced and beneficial to cancer cells. The mammalian target of rapamycin (mTOR) has been well discussed in its role to promote glycolysis; recent literature has revealed some new mechanisms of how glycolysis is promoted during skin cancer development (Figure 1).

On the other hand, Akt is not only involved in the regulation of mitochondrial metabolism in skin cancer but also of glycolysis. Activation of Akt has been found to phosphorylate FoxO3a, a downstream transcription factor of Akt, which promotes glycolysis by inhibiting apoptosis in melanoma. In addition, activated Akt is also associated with stabilized c-Myc and activation of mTOR, which both increase glycolysis for cancer cells [23,24].

Nevertheless, ras mutational activation prevails in skin cancer. Oncogenic ras induces glycolysis [25]. In human squamous cell carcinoma, the c-Jun NH(2)-terminal Kinase (JNK) is activated as a mediator of ras signaling, and is essential for ras-induced glycolysis, since pharmacological inhibitors if JNK suppress glycolysis [25].

CD147/basigin, a member of the immunoglobulin superfamily, is high expressed in melanoma and other cancers [26]. In a previous study, overexpression of CD147 promoted melanoma invasion, metastasis and angiogenesis, which is mediated via an interaction with the mediator of lactate transfer, Monocarboxylate Transporters 1 and 4 (MCT1 and 4), and increased glycolysis in A375 melanoma cells [27].

Glyoxalase I (GLO1) is a ubiquitous cellular defense enzyme involved in the detoxification of methylglyoxal, a cytotoxic byproduct of glycolysis. In human melanoma tissue, GLO1 is upregulated at both the mRNA and protein levels. Knockdown of GLO1 sensitizes A375 and G361 human metastatic melanoma cells to apoptosis. Therefore, GLO1 upregulation may play a functional role in glycolytic adaptations of cancer cells [28].

Since glucose uptake is facilitated by its transporters, the expression patterns of GLUT1 (glucose transporter 1) and GLUT4 (glucose transporter 4) in human oral carcinoma samples have been examined [29]. GLUT1 is expressed in the basal and parabasal layers of the different stratified squamous epithelia and in the cells of an oral carcinoma. However, GLUT4 is not expressed in any of the tissues examined. This result suggests GLUT1 might be the important transporter to facilitate glycolysis in skin epidermis.

The transcription factor HIF-1 upregulates a number of genes in low oxygen conditions including glycolytic enzymes, which promotes ATP synthesis in an oxygen independent manner. Studies have demonstrated that hypoxia induces HIF-1 overexpression and its transcriptional activity increases in parallel with the progression of many tumor types [30-32]. A recent study [33] demonstrated that in malignant melanoma cells, HIF-1 is upregulated, leading to elevated expression of Pyruvate Dehydrogenase Kinase 1 (PDK1), and downregulated mitochondrial oxygen consumption [34].

The M2 isoform of Pyruvate Kinase (PKM2), which is required for catalyzing the final step of aerobic glycolysis, is highly expressed in cancer cells; whereas the M1 isoform (PKM1) is expressed in normal cells. Our studies using the skin cell promotion model (JB6 cells) demonstrated that PKM2 is activated whereas PKM1 is inactivated upon tumor promoter treatment. Further studies suggest oxidative

stress, an important contributing factor in carcinogenesis, may play a critical role in PKM2 activation, since overexpression of MnSOD suppresses this event in the early stage of skin carcinogenesis [13]. However, oxidative stress signaling in general and oxidation of PKM2 itself may have a different impact on PKM2 activity. Dimitrios Anastasiou and colleagues [35] demonstrated that acute increases in ROS inhibited PKM2 through oxidation of Cys^{358} in human lung cancer cells. The levels of ROS and stage of tumor development may be pivotal for the role and regulation of PKM2.

Mitochondrial metabolism and glycolysis targeted skin cancer prevention and therapy

In cancer cells including skin cancer cells, the metabolic shift is composed of increased glycolysis, activation of anabolic pathways including amino acid and pentose phosphate production, and increased fatty acid biosynthesis. More and more studies have converged on particular glycolytic and mitochondrial metabolic targets for cancer drug discovery (Figure 2).

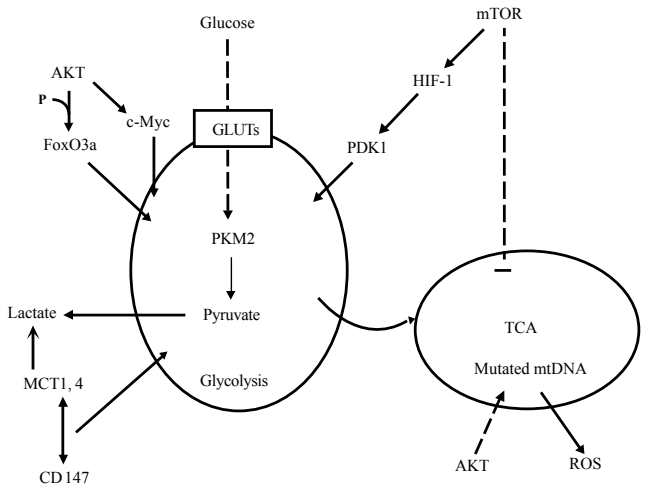

Figure 1: An overview of metabolism in skin cancer, Induction or increase, multistep process, repression.

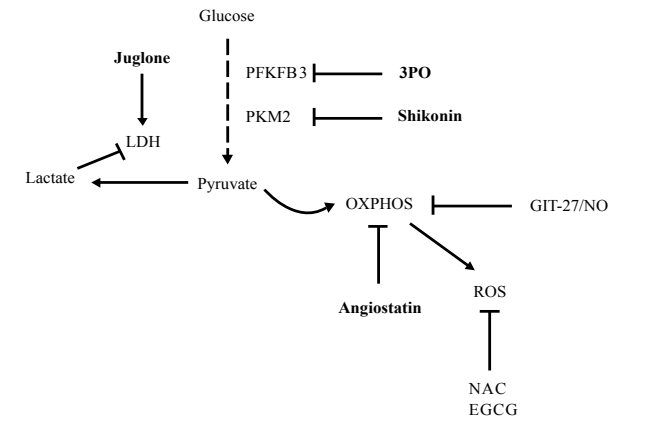

Figure 2: Chemicals target mitochondrial metabolism and/or glycolysis in skin cancer. Induction or increase, multistep process, repression.

A marker for increased glycolysis in melanoma is the elevated levels of Lactate Dehydrogenase (LDH) in the blood of patients with melanoma, which has proven to be an accurate predictor of prognosis and response to treatments. LDH converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent. High concentrations of lactate, in turn, negatively regulate LDH. Therefore, targeting acid excretion may provide a feasible and effective therapeutic approach for melanoma [36]. For instance, JugloSne, a main active component in walnut, has been used in traditional medicines. Studies have shown that Juglone causes cell membrane damage and increased LDH levels in a concentration-dependent manner in cultured melanoma cells [37].

As one of the rate-limiting enzyme of glycolysis, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase isozyme 3 (PFKFB3) is activated in neoplastic cells. Studies have confirmed that an inhibitor of PFKFB3, 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO), suppresses glycolysis in neoplastic cells [38]. In melanoma cell lines, the concentrations of Fru-2, 6-BP, lactate, ATP, NAD^+, and NADH are diminished by 3PO. Therefore, targeting PFKFB3 using 3PO and other PFKFB3 specific inhibitors could be effective in melanoma chemotherapy [38].

A new NO (nitric oxide) donating compound [(S,R)-3-phenyl-4,5-dihydro-5-isoxazole acetic acid–nitric oxide (GIT-27NO)] has been tested in treating melanoma cells. The results suggest that GIT-27/NO causes a dose-dependent reduction of mitochondrial respiration in treated A375 human melanoma cells [39].

As an endogenous angiogenesis inhibitor, angiostatin is produced by autoproteolytic cleavage of plasminogen. Currently, the molecular mechanism of its anticancer function is being extensively studied. In human melanoma tumor cells (A2058), it has been reported that at least two mitochondrial enzymes are affected by angiostatin [40] which include malate dehydrogenase, a member of the Kreb's cycle enzymes; and adenosine triphosphate synthase. Both are identified potential angiostatin-binding partners. After treated with angiostatin, the ATP concentrations of A2058 cells were decreased. Meanwhile, using siRNA of these two enzymes also inhibited the ATP production. Therefore, angiostatin has great potential to become an anticancer medicine (agent).

UV is a potent inducer for ROS generation in the skin. The antioxidant N-acetylcysteine (NAC) has be used in patients before being exposed to UV. It was shown that NAC could prevent pro-oncogenic oxidative stress and reduce melanoma risk [41]. As an antioxidant constituent in green tea, (-)-epigallocatechin-3-gallate (EGCG) inhibited the viability and growth of melanoma and promoted apoptosis, with the cki-cdk-cyclin network and Bcl2 family proteins contributing to this process [42].

Our recent studies demonstrated that PKM2 is up regulated in the early stage of skin carcinogenesis [13], therefore, targeting PKM2 could serve as a new approach for skin cancer prevention and therapy. There are two PKM2 inhibitors that have been reported, compound-3 (*N*-(3-carboxy-4-hydroxy) phenyl-2,5,-dimethylpyrrole) [43] and shikonin [3], that both show more specificity to PKM2 than PKM1. Inhibiting PKM2 using these inhibitors may provide a new strategy for developing preventative and anticancer therapies.

Conclusion

As reported by the National Cancer Institute (NCI), more than

68,000 Americans are diagnosed with melanoma, and more than 2 million with basal cell or squamous cell skin cancer each year. Therefore, accurate diagnosis and aggressive treatment play an important role in the control of skin cancer.

Recently the "Warburg Effect" in skin cancer and how to target it for skin cancer prevention and therapy has attracted more attention. While whether mitochondrial metabolism and biogenesis are up- or down-regulated in skin cancer remains controversial and further efforts are required to clarify this, increased glycolysis seems well observed and the signaling pathways critical for this activation could serve as novel preventive and therapeutic targets for human skin cancer.

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