Regulation of glycolysis in head and neck squamous cell carcinoma

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Abstract Glycolysis is highly upregulated in head and neck squamous cell carcinoma (HNSCC). HNSCC glycolysis is an important contributor to disease progression and decreases sensitivity to radiation or chemotherapy. Despite therapeutic advances, the survival rates for HNSCC patients remain low. Understanding glycolysis regulation in HNSCC will facilitate the development of effective therapeutic strategies for this disease. In this review, we will evaluate the regulation of altered HNSCC glycolysis and possible therapeutic approaches by targeting glycolytic pathways.

Keywords: HNSCC, glycolysis, p53, HIF-1, GLUT-1, HK-II and LDH-A

Background

Head and neck squamous cell carcinoma (HNSCC) is the most common type of head and neck cancer [1-3]. HNSCC is the 6th most common cancer worldwide with the incidence of 650,000 new cases every year [4]. It arises from the mucosal lining of the oral cavity, larynx, pharynx, oropharynx, hypopharynx, sinonasal tract and nasopharynx [1]. The major risk factors for HNSCC are tobacco and alcohol exposure [5-8], and human papilloma virus (HPV) infection [9-11]. These factors are associated with the HNSCC progression [7, 12].

Cancer cells are dependent of several metabolic processes including glycolysis [13], mitochondrial oxidative phosphorylation (OXPHOS) [14], and glutaminolysis to fulfil their energy requirements [15]. Like most aggressive tumors, HNSCC exhibit a high rate of glycolysis to meet their metabolic demands [16, 17]. The outcome of the increased glycolysis in tumors can be visualized by (18F) fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET)/computed tomography (CT) (FDG-PET/CT) using a glucose analog, FDG as a tracer [18-20]. The high FDG uptake by the HNSCC tumors potentially correlates with the glucose uptake by the cells. Moreover, the molecular imaging studies of HNSCC using FDG-PET/CT imaging demonstrated the high glucose uptake and increased glycolysis in HNSCC [21-23].

Glycolysis is a pathway that serves as the foundation for cellular metabolism [24, 25]. Glycolysis regulation in HNSCC is associated with the alteration in oncogenes, tumor suppressor genes, overexpression of glycolytic enzymes and glucose transporter. Under aerobic condition, cells produce only two adenosine triphosphates (ATPs) through glycolysis. Whereas, mitochondrial respiration can produces 36 ATPs by utilizing a product of glycolysis, pyruvate through OXPHOS. Under anaerobic conditions, pyruvate is reduced to lactate by an enzyme, lactate dehydrogenase A (LDH-A). Although, glycolysis generates less energy, ATP than OXPHOS, glycolysis is a major characteristic of cancer cell metabolism. Because of the weakened OXPHOS and less utilization of pyruvate, cancer cells produce less ATP through OXPHOS. In order to maintain a balance of energy, cancer cells aggressively perform glycolysis. The fast generation of energy during glycolysis promotes cell proliferation in rapidly growing cancer cells including HNSCC [13, 26, 27].

There are several steps involved in glycolysis to produce two molecules of pyruvate from one glucose molecule. Briefly, glucose first phosphorylates into glucose-6-phosphate by a catalytic enzyme, hexokinase. Next, glucose 6-phosphate converts into fructose 6-phosphate, fructose 6-phosphate converts into fructose 1,6-bisphosphate and finally it produces pyruvate as a end product of glycolysis [28]. In cancer cells, the elevated LDH-A catalyzes most of the pyruvates into lactate. The intracellular accumulation of lactate is extremely harmful as
the abundance of lactate drastically lowers intracellular pH. Consequently, the export of lactate by lactate transporters (Monocarboxylate transport proteins (MCTs)) into the extracellular space is extremely important for the cancer cells to main their intracellular pH.

The purpose of this review is to highlight the current studies of HNSCC glycolysis and possible therapeutic interventions.

**Regulation of HNSCC glycolysis**

In 1927, Otto Warburg demonstrated that human tumor cells preferentially utilize glycolysis to generate energy using *in vivo* and *in vitro* models [29]. Warburg demonstrated that although mitochondrial respiration produces more ATP, tumors unlike normal cells, preferentially engage in glycolysis even in the presence of oxygen. Later, this phenomenon came to be known as the Warburg effect or aerobic glycolysis [30]. Increased aerobic glycolysis in cancer cells favorably accumulates lactic acid in tumor microenvironment. The accumulation of high amount of lactic acid tumor microenvironment is associated with metastatic spread and radioresistance in HNSCC [31, 32].

The underlying mechanisms involved in the preferential use of glycolysis by cancer cells include mitochondrial defects, adaptation to hypoxic tumor microenvironments, oncogenic signaling and abnormal expression of metabolic enzymes [33]. Several studies have demonstrated that altered glycolysis in HNSCC is associated with the activation of hypoxia-inducible factor-1 (HIF-1), transketolase-like protein 1 (TKTL1), mutations in tumor suppressor gene, p53, as well as overexpression of the glucose transporters-1 (GLUT-1), and the glycolytic enzymes, Hexokinase-II (HK-II), lactate dehydrogenase A (LDH-A) and monocarboxylate transport proteins (MCTs) (Figure 1) [13, 34-38]. In the next section, we will discuss the role of each of these molecules in HNSCC pathogenesis.

**Hypoxia-inducible factor-1**

Hypoxia-inducible factor-1 (HIF-1) is a transcription factor, which reprograms and regulates cancer cell metabolism including aerobic/anaerobic glycolysis and lactate production [39, 40]. HIF-1 is a heterodimer consisting of a highly regulated HIF-1α subunit and a constitutively expressed HIF-1β subunit [41-43]. A high level of HIF-1α protein is common in many types of solid tumors including head and neck [44-47]. Recently, it has been reported that HIF-1α complex binds hypoxia response elements in the promoter region of target genes, which include enzymes involved in glycolysis and pH regulation, such as phosphoglycerate kinase [48], carbonic anhydrase CA9 [49] , hexokinase [50], lactate dehydrogenase [51] and glucose transporters, GLUT-1 and GLUT-3 [41, 52-54].

HIF-1 increases the rate of glucose uptake in cancer cells through the induction of glucose transporters and further induces glucose phosphorylation by increasing HK-II activity [50]. HIF-1 can regulate complete glycolysis pathway by increasing the amounts of the enzymes (e.g. HK-II, LDH-A and phosphofructokinase1 (PFK1)) involved in this process [55]. Seagroves et al. (2001) reported that most of the enzymes necessary for glycolysis in mammalian cells are regulated by HIF-1 [56]. Moreover, the overexpression of HIF-1 promotes the expression of glycolytic enzymes, which favors the use of glucose in glycolysis and lactate export into extracellular space.

**Transketolase-like protein 1**
Transketolase-like protein 1 (TKTL1) is a heterodimer protein belonging to the transketolase family [57]. The overexpression of TKTL1 is associated with the cell growth, glucose consumption and lactate production in many cancer cells and is considered as a potential proto-oncogene [58-61]. In 2009, Smith and coworkers reported that the TKTL1 induced aerobic glycolytic phenotype in head and neck and lung cancer by increasing fructose-6-phosphate and glyceraldehyde-3-phosphate [61]. Moreover, the overexpression of TKTL1 is strongly correlated with tumor progression in colorectal cancer [62]. The function of TKTL1 is not very well understood in HNSCC. Recently, Grimm et al. (2014) demonstrated that the overexpression of TKTL1 is negatively correlated with the survival of patients with OSCC [63]. In 2010, Sun et al. reported the overexpression of TKTL1 in HNSCC tumors compared to normal mucosa [64]. Moreover, the overexpression of TKTL1 in HNSCC cells promoted cellular proliferation in vitro and tumor growth in vivo [64]. The overexpression of TKTL1 increased the production of fructose-6-phosphate and glyceraldehyde-3-phosphate, and further increased lactate production by stabilizing HIF1α (Figure 1) [64].

**Figure 1: Regulation of HNSCC glycolysis.** HNSCC tumors are more dependent on glycolysis. This model represents the regulation of glycolysis in HNSCC. Mutation in p53 activates HIF-1α, which regulates GLUT-1, HK-II and LDH-A expression in HNSCC. The overexpression of TKTL1 in HNSCC increases lactate production by increasing fructose-6-phosphate and glyceraldehyde-3-phosphate activity. Overexpression of MCTs export lactate out from the cells to protect cell damage as of the high accumulation of lactate decreases pH in intracellular environment.
**p53**

*p53* is a tumor suppressor gene, which is highly mutated in human cancers [65]. The *p53* signaling pathway is activated in response to a variety of stress signals, allowing *p53* to coordinate transcription programs that ultimately contribute to tumor suppression [66]. *p53* also regulates cellular metabolism by regulating glycolysis and OXPHOS steps via transcriptional regulation of its downstream genes TP53-induced glycolysis regulator (TIGAR) [67]. Loss or gain of function, through mutations in *p53* (Mut-*p53*) is a common feature in the majority of human cancers [68, 69]. Recently, it has been reported that the gain-of-function in *p53* mediates metabolic changes in tumors and promotes tumor growth [70]. Zhang et al. (2013) demonstrated that Mut-*p53*, gain of function promoted glycolysis and Warburg effect in several cancers by increasing GLUT-1 expression through activating RhoA/ROCK signaling pathway in both *in vitro* and *in vivo* models [71]. Moreover, the Mut-*p53* was reported to induce the expression of glycolytic enzyme, HK-II, which promoted glycolysis in rats [72]. Although, mutations in *p53* is a key factor in the regulation of cancer cell metabolism, the inactivation of *p53* in HPV positive HNSCC indicates an alternative mechanisms of HNSCC metabolic regulation [73-75]. Currently, the roles of Mut-*p53* in regulation of tumor metabolism remain unclear. Whereas, some studies have demonstrated that glycolytic inhibition potentiates radiation toxicity in Mut-*p53*, but not in *WT-p53* expressing HNSCC cells [68, 76].

**Glucose transporter-1**

Glucose transporter-1 (GLUT-1) encoded by a gene called SLC2A1, which mediates the cellular uptake of glucose into many tissues, and maintains glucose concentration in blood [77-79]. Overexpression of GLUT-1 was reported in several cancers including head and neck [80-82]. In 2008, Li et al. reported the overexpression of GLUT-1 in primary and recurrent HNSCC tumors with the high accumulation of FDG [83], which correlates with the high glucose uptake and poor patients survival. These findings indicate that the overexpression of GLUT-1 in HNSCC is associated with glucose uptake.

**Hexokinase-II**

Hexokinase-II (HK-II) is a glycolytic enzyme, which phosphorylates glucose into glucose-6-phosphate in many tissues including muscle and adipose [84, 85]. HK-II is one of the rate-limiting enzymes of glycolysis [13], which is highly upregulated in many cancers including HNSCC [63, 86-88]. Recently, Chen et al. (2014) reported the overexpression of HK-II in laryngeal squamous cell carcinoma (LSCC) cells and the effect of HK-II expression in tumor progression. HK-II knocked down by HK-II shRNA in laryngeal cell carcinoma, Hep-2, decreased cell viability and increased apoptosis by arresting GO-G1 phase of cell cycle. Moreover, the depletion of HK-II resulted in reduced xenograft tumor [89]. These findings suggest that the HK-II expression in HNSCC plays an important role in the tumor progression by upregulating tumor glycolysis.

**Lactate dehydrogenase A**

The lactate dehydrogenase A (LDH-A) is an enzyme, which is highly upregulated in cancer cells. LDH-A catalyzes the last step of anaerobic glycolysis, converts pyruvate into lactate during glycolysis [90, 91]. There are five active LDH isoenzymes in human tissue, each of which is a tetrametric metabolic enzyme composed of two major subunits, A and B, encoded by *Ldh-A* and *Ldh-B* genes, respectively. When there are more A chains than B chains, the LDH isoenzymes become more efficient in catalyzing the conversion of pyruvate into lactate; conversely, an excess of B chains favors the conversion of pyruvate into acetyl-CoA. The dependence of tumor cells on LDH-A has been demonstrated in many cancer types including OSCC [63, 92]. During the conversion of pyruvate into lactate, nicotinamide adenine dinucleotide (NADH) reduced to NAD+ ion,
which required to drive glycolysis in cancer cells. LDH-A is a key enzyme involved in the Warburg effect and in sustaining cancer’s glycolytic phenotype [93]. Recently, Blatt et al., (2016) demonstrated that the high lactate levels in HNSCC tumors are significantly negatively correlated with patient survival [94].

Monocarboxylate transports

Most solid tumors are highly glycolytic, which produce significant amount of lactic acid in extracellular environment, contributing to the acidic tumor microenvironment [95]. In this condition, monocarboxylate transports (MCTs) play an important role in the maintenance of the lactate shuttle, and pH regulation in highly glycolytic solid tumors [96]. MCTs are transmembrane proteins that facilitate the transport of variety of substrates such as pyruvate and lactate [97]. The overexpression of monocarboxylate transports (MCT1 and MCT4) have been reported in several cancers including head and neck cancer and is associated with the poor prognosis [27, 96, 98-100]. The regulation of MCT1 and MCT4 depends on the specific cell types and their function [101]. Several studies have demonstrated that MCT1 works as a lactate bidirectional transporter, whereas MCT4 only effluxes lactate from the cells [102, 103]. In solid tumors, cancer cells could also import lactate through MCT1 from the most glycolytic tumor cells [103, 104] or tumor-associated fibroblasts to fuel mitochondrial respiration and thereby spare glucose for hypoxic tumor cells [105, 106]. In 2011, Boidot et al. demonstrated that loss of p53 in hypoxia condition induces MCT1 expression and increases lactate shuttle in elevated glycolytic tumors [107]. As discussed above, the intracellular accumulation of lactate is tremendously harmful for the cancer cells in highly glycolytic solid tumor. Thus, the export of lactate into the extracellular space through MCTs is extremely necessary for the survival of cancer cells. Therefore, targeting MCTs in highly glycolytic HNSCC tumors could be a potential therapeutic approach to control tumor growth [95, 108, 109].

Therapeutic approaches for HNSCC by targeting glycolytic pathways

Despite development of several metabolic inhibitors, not many studies have been reported to target glycolytic pathway in HNSCC. As HK-II phosphorylates glucose to glucose-6-phosphate, most of the glycolysis inhibitors were designed to target HK-II. Ionidamine, 2-deoxy-D-glucose (2-DG) and 3-bromopyruvate (3-BP) are the most commonly tested HK-II inhibitors being used in both pre-clinical and clinical model either alone or in combination with chemotherapy and radiation therapy [33, 110-114]. Simons et al., (2007) demonstrated that 2-DG potentiates cisplatin cytotoxicity in HNSCC xenografts model [111, 112]. In cancer cells, glucose deprivation as well as treatment with 2-DG has been shown to induce oxidative stress and sensitivity to radiation and chemotherapy [115-118]. In 2008, Ihrlund et al. demonstrated that 3-BP enhanced cisplatin cytotoxicity in pre-clinical setting [119]. Other clinical studies demonstrated that the combination of lonidamine to either radiation therapy or chemotherapy improved clinical outcomes in HNSCC patients [120, 121].

As GLUT-1 increases glucose uptake in HNSCC and potentiates glycolysis, it is important to target GLUT-1 to inhibit glycolysis. Most recently, Wang et al. (2013) demonstrated that the inhibition of GLUT-1 activity and expression can sensitize HNSCC cells to cisplatin treatment in both in vitro and in vivo models. They demonstrated that glucose uptake was reduced in HNSCC cells by knocking down GLUT-1 with shRNA or blocking GLUT-1 by anti-GLUT-1 antibody. Both anti-GLUT-1 antibody and GLUT-1-shRNA sensitized HNSCC cells to cisplatin treatment under both normoxia and hypoxia conditions [122]. Another study by Li et al. (2013) demonstrated that the inhibition of GLUT-1 in HNSCC significantly inhibited cell viability and colony formation. Further, GLUT-1 inhibition reduced tumor growth in xenograft model [123]. In addition to HK-II and GLUT-1, HIF-1α, p53, TKTL1, LDH-A and MCTs can also
be targeted to inhibit glycolytic pathway in HNSCC.

**Conclusion**

In this review, we discussed the role of HIF-1, TKTL1, p53, GLUT-1, HK-II, LDH-A and MCTs in HNSCC metabolism. In general, expression of these factors was associated with poor survival, and resistance to radiation and chemotherapeutic agents in HNSCC. The effect of these markers in HNSCC glycolysis is not very well investigated. Therefore, the detailed study and evaluation of these factors in HNSCC glycolysis may provide clues for the best treatment option for HNSCC patients with radio or chemo resistance.

**Acknowledgments**

This work was partially supported by National Institute of General Medical Sciences, National Institute of Health (P20 GM103418) funded KINBRE postdoc fellowship.

**Conflict of Interest**

The author declares no competing interests.

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