10-2020

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PII: S0024-3205(20)30988-7

DOI: https://doi.org/10.1016/j.lfs.2020.118236

Reference: LFS 118236

To appear in: *Life Sciences*

Received date: 22 May 2020

Revised date: 25 July 2020

Accepted date: 5 August 2020

Please cite this article as: V. Chandel, S. Raj, P. Kumar, et al., Metabolic regulation in HPV associated head and neck squamous cell carcinoma, *Life Sciences* (2018), https://doi.org/10.1016/j.lfs.2020.118236

Metabolic regulation in HPV associated head and neck squamous cell carcinoma

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Abstract

Cancer cells exhibit distinct energy metabolic pathways due to multiple oncogenic events. In normoxia condition, the anaerobic glycolysis (Warburg effect) is highly observed in Head and Neck Squamous Cell Carcinoma (HNSCC). HNSCC is associated with smoking, chewing tobacco, consumption of alcohol or Human Papillomavirus (HPV) infection primarily HPV16. In recent years, the correlation of HPV with HNSCC has significantly expanded. Despite the recent advancement in therapeutic approaches, the rate of HPV infected HNSCC has significantly increased in the last few years, specifically, in lower middle-income countries. The oncoproteins of High-risk Human Papillomavirus (HR-HPV), E6 and E7, alter the metabolic phenotype in HNSCC, which is distinct from non-HPV associated HNSCC. These oncoproteins, modulate the cell cycle and metabolic signaling through interacting with tumor suppressor proteins, p53 and pRb. Since, metabolic alteration represents a major hallmark for tumorigenesis, HPV acts as a source of biomarker linked to cancer progression in HNSCC. The dependency of cancer cells to specific nutrients and alteration of various metabolic associated genes may provide a unique opportunity for pharmacological intervention in HPV infected HNSCC. In this review, we have discussed the molecular mechanism(s) and metabolic regulation in HNSCC depending on the HPV status. We have also discussed the possible potential therapeutic approaches for HPV associated HNSCC through targeting metabolic pathways.

Keywords: HNSCC; HPV Infection; Glycolysis; Metabolism; Cancer therapy

1. Introduction

Despite the fact that Warburg phenomenon was first described centuries ago, however, the association between major metabolism regulators and oncogenes has fueled a renewed interest in the area of cancer cell metabolism in the last few years [1]. Although, every year with the incidence of 650,000 new cases, head and neck squamous cell carcinoma (HNSCC) has been recognized the sixth most common cancer worldwide [2]. HNSCC is classified into two subgroups, Human Papillomavirus negative (HPV^-) and Human Papillomavirus positive (HPV^+) HNSCC [3]. The most common cause of HNSCC malignancies includes smoking chewing tobacco and alcohol consumption with the case of HPV^- HNSCC and Human Papillomavirus in HPV^+ HNSCC (Fig.1) [4]. HPV^+ HNSCC are commonly associated with oropharyngeal part of the Head and Neck, usually determined by the expression of p16 (surrogate marker) [5]. In corresponding to migration, invasion, and response to chemotherapy, HPV infected HNSCC has different clinical behavior, molecular and metabolic profiles [6]. The routine technique used for assessing HNSCC tumor is PET-CT scan imaging, which state the metabolic activity of the cells [7,8]. This suggests that a specific metabolic pathway may facilitate the promotion and progression of HNSCC tumor and can represent therapeutic targets. It has been reported that HPV^+ HNSCC cells rely on mitochondrial respiration with decreased rate of glycolysis, whereas, HPV^- HNSCC cells depend on glucose metabolism for survival [9]. HNSCC meet their metabolic demands highly depending on the anaerobic glycolysis that helps cancer cells in rapid proliferation [10]. HNSCC cells with HPV^+ infection gets benefited hugely by glycolysis independently of the oxygen availability where it helps the HNSCC to resist the chemotherapeutic drug as well as detoxify the free radicals. Additionally, glycolytic regulation also produces pyruvate and lactate, which promote and confer radio resistance [11]. This review primarily focusses on the basic principle and mechanism(s) of cancer cell metabolism in HPV associated HNSCC, showing differential therapeutic outcome,

which may lead to the altered metabolic activity and possible intervention(s) for the effective treatment against different subtype of HNSCC.

2. HPV in head and neck cancer

Human Papillomavirus (HPV) is a double-stranded, non-enveloped DNA virus consisting of approximately 8000 base pairs and encoding a total of 8-9 proteins. More than 100 distinct HPV genotypes are identified till date [12]. The E6 and E7 HPV oncoproteins cooperatively promote loss of cell cycle control, inhibit apoptosis, promote uncontrolled cell proliferation and induce chromosomal instability through degrading two major tumor suppressor genes, p53 and pRb [13,14]. It has become clear over the last few decades that HPV not only causes cervical cancer, but also causes a subset of HNSCC [15]. HNSCC patients with HPV infection are reportedly younger in age and also have different clinical characteristics where tobacco remains a contributor to the disease. [16]. HPV^+ HNSCC as compared to the HPV^- HNSCC samples have poorly characterized histology. Additionally, the HPV^+ tumors are tend to be diagnosed at small tumor stage. Interestingly, patients with HPV^+ status show improved performance rate and a better prognosis as compared to HPV^- patients (Fig.1). The continuous increase in HPV infected HNSCC cases in the last few years, is picking up people's attention globally. The epidemiology of HPV associated HNSCC is largely relying on the tumor subset and the region [17].

Among the report of 500,000 annual cases of HNSCC disease a approx. number of 85,000 is reported to be HPV^+ which suggests that most common HPV infected tumor site is of head and neck region [18]. Studies show that HPV infection causes about 30–60% of oropharyngeal carcinoma, 12% of pharyngeal cancer and 3% of oral cancer [3]. Oropharyngeal cancer shows the most accepted site for HPV associated tumors after cervical cancer as compared to the other tumor sites of oral cavity (12%) and the oropharynx (14%) (Fig.1) [19,20]. HPV associated oropharyngeal carcinoma occurs primarily in the tonsils, back of the tongue, or in the palatine region. Recent study suggests that 62% of the tongue based tumors are HPV^+, whereas 25% are HPV^- [21].

3. Genetic alterations in HPV$^+$ and HPV$^-$ head and neck cancer

HPV$^+$ and HPV$^-$ HNSCC present differences in terms of epidemiology, etiology, genetic alterations, metabolism, molecular properties and response to the treatment [22]. p53 and pRb pathways are frequently altered and inactivated in both HPV$^+$ and HPV$^-$ HNSCC. While the inactivation mechanism of both p53 and pRb remains quite different [23]. As described above, the inactivation of p53 and pRb is caused by E6 and E7 oncoproteins in HPV$^+$ HNSCC. In contrast, p53 is highly mutated in HPV$^-$ HNSCC [18,24]. The higher mutation rate in HPV$^-$ HNSCC has been reported in several studies as compared to HPV$^+$ HNSCC [25,26]. Other studies have reported that there is a difference in genomic aberration profile and not the mutation rate in both groups. For instance, HPV$^+$ HNSCC has a higher number of mutations in genes, such as TRAF3, FGFR2/3, PIK3CA, MLL2/3, DDX3X, KRAS, E2F1; while in HPV$^-$ HNSCC, TP53, MLL2, NSD1, EGFR, EPHA2, NOTCH, PIK3CA, FGFR2/3, FGFR, CUL3, CDKN2A, DDR2, CASP8, HRAS genes are mutated [27]. Certain genetic modifications are shared in HNSCC irrespective of the HPV status; however, others have been specifically related to either HPV$^+$ or HPV$^-$ HNSCC [28]. Comparative genomic and sequencing studies have shown the amplifications in chromosome 3q, 8q, and 20p in HNSCC. Also, chromosome arms 3p and 9p are frequently lost, whereas, 11q13 is highly amplified in HPV$^-$ HNSCC [29]. Several genes like SOX2, PIK3CA and TP63 are located in the 3q26-28 region [30]. CDKN2A and CCND1 encoding p16^{INK4A} and Cyclin D1 are located on 9p and 11q13 are associated in pRb signaling and therefore the success rate of modification is less in HPV associated HNSCC [31]. Similarly, HPV infected HNSCC samples reportedly have TP53 mutations widely while not much in HPV$^-$ HNSCC [32]. In addition, EGFR and FGFR1 amplifications are observed specifically in HPV$^+$ HNSCC. Contrary, 14% of deletion or 8 % of mutation has been reported in receptor-associated factor 3 (TRAF3), which is mainly involved in the innate and acquired antiviral immune response, is overexpressed in HPV$^+$ HNSCC [33]. Studies in HPV$^+$ HNSCC have reported

elevated levels of TpC mutation frequencies and CpG transversions in HPV$^-$ HNSCC, whereas the presence of HPV infection quite likely does not affect the overall mutation rate [34].

4. Cellular metabolism in HPV infected HNSCC

Adenosine triphosphate (ATP) provide energy to the cells and that utilize through carbohydrate, protein and fat metabolism [35]. End products such as the pyruvate and acetyl coenzyme-A is the result of metabolism of glucose, amino acids and other intermediaries, such as glycerol and fatty acids. The subsequent metabolic activity of acetyl-CoA in the Tricarboxylic acid cycle (TCA) and OXPHOS cycle results in the generation of energy products such as flavin adenine dinucleotide (FADH2), nicotinamide adenine dinucleotide (NADH), and ATP [36]. Cancer cells undergo metabolic reprogramming to obtain their energy requirements since their energy requirements differ from those of normal cells (Fig.2) [37].

4. 1. Glycolysis

As compared to normal cells, glycolysis is highly upregulated in HNSCC, despite in the existence of oxygen [1]. The alteration in the levels of expression and regulation of certain tumor suppressor genes, oncogenes, and various other glycolytic enzymes and transporters determines the regulation of glycolysis in HNSCC [38]. During aerobic condition, the cells produce two ATPs with the help of glycolysis. Whereas, TCA cycle produces 36 ATPs by utilizing pyruvate through OXPHOS. Under aerobic conditions pyruvate has the fate to produce lactate with the help of the enzyme lactate dehydrogenase (LDH-A). Apart from the less production of energy in the form of ATP glycolysis has been stated to be a major feature for cancer cell metabolism [39]. Subsequently the less utilization of pyruvate and deteriorated OXPHOS, cancer cells produce less ATP through OXPHOS. Cancer cells perform glycolysis aggressively in order to maintain energy balance. The rapid generation of energy during glycolysis promotes aggressive proliferation of HNSCC [4,36]. Certain evidence suggests the involvement of HPV in metabolic reprogramming of HNSCC.

Interaction of E6 and E7 viral oncoproteins with several cellular partners have been reported to affect biological functions [40]. Studies have also shown the association between HR- HPV, E6 & E7 and Hypoxia-inducible factor-1 (HIF-1) and its downstream gene expression [41]. HIF-1α is a transcription factor responsible for regulating and reprogramming cancer cell metabolism, enabling tumor cells to survive via modulating glycolysis and pentose phosphate pathway (PPP). This helps in inducing angiogenesis, cell proliferation, erythropoiesis, invasion and apoptosis [42,43]. Overexpression of HIF-1α has been reported in several solid tumors, including HNSCC [44,45]. HIF-1 is a heterodimer protein which contains two subunits namely HIF-1α mostly regulated by oxygen and a constitutively expressing HIF-1β subunit [9]. The protein family, prolyl hydroxylase domain (PHD) mainly functions by stabilising both the subunits of HIF-1 [11]. However, HIF-1α is rapidly weaken under normoxia condition, because HIF-1α hydroxylated by PHD2, which discover a binding site for von Hippel-Lindau (VHL) which is a tumor suppressor protein. Further, E3 ubiquitin ligase complex promotes fast deterioration of HIF-1α [46]. Under hypoxic condition, a complex formation of HR-HPV, E6 & E7 with HIF-1α and the PHD activity is prohibited by mutated VHL. This also detoriates the proteasomal deterioration of HIF- 1α via ROS and NOS which accumulates and stabilize the HIF-1α subunit [47]. After HIF-1α complex is stabilized this subunit specifically binds to the hypoxia response elements (HRE), which may present in the promoter site of target genes. This stimulates transcription of multiple genes, which has been associated within glucose metabolism, such as hexokinase (Fig.3) [48], glucose transporters (GLUT1 and GLUT3) [49.50], lactate dehydrogenase (LDH) [51], phosphoglycerate kinase [52], Na+ve/H+ve exchanger 1 (NHE1) and carbonic anhydrase IX (CAIX) [11].

HNSCC associated with HPV has favorable outcomes as compared to the HPV^- [53]. It has been observed that in HPV^+ HNSCC, a differentially regulated metabolic machinery is shown to play major role [54]. Additionally, HIF-1α has been reported as an important hypoxia biomarker in radioresistance and crucial metabolic regulator in cancer cells [40]. In 2017, Jennifer et al., have

reported the higher expression of HIF-1α in HPV^+ HNSCC as compared to HPV^- in normoxia condition [55]. This was followed by the correlation between HPV16-E7 and HIF-1α. This suggests that there is a positive association between the expression of HPV and HIF-1α. Under hypoxic condition, upregulation of HIF-1α in both HPV^+ and HPV^- HNSCC cells has been reported. However, the absolute increase in protein and hypoxia expression signal was higher in HPV^+ cells as compared to HPV^- [41]. HIF-1α activates transcription of glycolytic enzymes as well as glucose transporters, leading to an aggressive tumor behavior [56]. Rodolico et al., in 2011 stated in his findings that there is a steady linkage between the formulation of HPV16-E7 and HIF-1 in HPV16 infected oral squamous cell carcinoma tissue specimens collected from 62 randomly selected patients who underwent surgical resection [57]. As discussed above, HIF-1α binding to the HRE promoter region stimulates the transcription of multiple genes associated with glucose metabolism. HIF-1α increases the expression of glucose transporters. This leads to an increase in glucose uptake and induces phosphorylation of glucose by increasing the activity of HK-II activity [58]. The entire pathway of glycolysis is regulated by HIF-1α via upregulating the major enzymes responsible for glycolysis such as HK-II, LDH-A and phosphofructokinase1 (PFK1). Furthermore, upregulation of these glycolytic enzymes, favours the utilisation of glucose in glycolysis and export of lactate into the extracellular space. This promotes the tumor cell angiogenesis and survival [59]. Hence, HIF-1α is a major pathway causing metabolic alteration in HNSCC. Because under normoxia, non-metastatic HNSCC cells utilise less glucose and HIF-1α, suggesting that HIF-1α dysregulation may induce warburg effect. The development of [^{18}F]fluorodeoxyglucose positron emission tomography imaging (FDG-PET), co-ordinated with recent findings has been shown to have been associated with aggresive cancer phenotypes [60]. Therefore, it is very important to investigate the role of HIF pathway in HPV^+ associated HNSCC to provide an insight for the development of potential therapeutic strategies.

p53 is a tumor suppressor gene and is found to be mutated in various cancers, including HNSCC. Also, p53 is reported to be a major regulator of metabolic mechanism such as glycolysis and OXPHOS [61]. Degradation of p53 by HR- HPV-E6, mediates metabolic reprograming in HNSCC [15]. The highly important cell nutrients, which may synthesize carbon sources and macromolecules in the cells, do not freely diffuse through the lipid bilayer and are hydrophilic. These molecules need specific transporters present in the cell membrane to pass through. For instance, GLUT facilitate the entry of glucose in the cell [62,63]. Additionally, oncogenes including RAS, Myc and SRC induce the overexpression of GLUT1 and GLUT3 to maintain the Warburg phenomenon and mediate the entry of glucose in the cell (Fig.5) [64]. GLUT-1 upregulation provides several metabolic advantages to cancer the cells helping them into energy and biomass production for their survival. Also, GLUT-1 expression leads to the production several glycolytic intermediates which are further utilised by other metabolic pathways such as PPP, nucleotide production or synthesis of lipid [64] A huge interpretation of GLUTs has been shown in the majority of cancers including HNSCC. The transcriptional regulation of p53, represses GLUT1 and GLUT4 indirectly, and limiting the uptake of glucose in the cancer cells, therefore, weaken their growth [65]. HR-HPV-E6 mediated degradation of p53, upregulates the expression of GLUT1 & GLUT4, and therefore elevate glucose uptake [66]. SGLT Na^+ /glucose co-transporters through Na^{+ve} /K^{+ve} ATPase secondary active transport mediate glucose internalization in epithelial cells, which is coupled to Na^{+ve} entry. The low concentrations of glucose, SGLT1 Na^{+ve}/glucose co-transporters present in HPV^+ HNSCC facilitates glucose accumulation [67]. HPV^+ HNSCC shows significant over-expression of GLUT1 in HNSCC, correlating with the highest accumulation of FDG and thus higher uptake of glucose (Fig. 4). However, HPV^+ patients have a significantly better disease free survival rate as compared with the HPV^- HNSCC patients [68]. GLUT1 upregulation in HPV^+ tumors has been observed in central locations. In HPV^- tumors, the expression of GLUT1 was either indifferent within the nests of solid tumor or overexpression was observed at the

margins, and decreased in central location [69]. The mRNA expression level of GLUT1 was significantly higher in HPV^+ HNSCC cells as compared to HPV^-. Additionally, Warburg effect is enhanced by HPV16-E6 & E7 oncoproteins, which interact with HIF-1α and promote its degradation by VHL, which significantly upregulates the expression of GLUT1 [47].

Hexokinase-II (HK-II) is a rate limiting enzyme of glycolysis, converts glucose to glucose-6-phosphate (G6P) in many tissues, such as adipose and muscle [70]. The regulation of glycolysis by HPV mediated upregulation of HK-II, facilitates the promotion of cancer cell survival, associated with poor prognosis [71,72]. Four isoforms of HK has been reported, among which the upregulation of HK-II is highly observed in the majority of cancers including HNSCC. HK-II mediates the stimulation of aerobic glycolysis and plays a major role in generating building blocks for tumor cell growth [10]. Additionally, HK-II supplies intermediate metabolites and stimulates TCA cycle by utilizing glutamine derived carbon in anaplerosis [73]. Moreover, HK- II regulates autophagy by suppressing proapoptotic molecules Bax and Bad at the outer mitochondrial membrane. It plays a critical role in metabolism through integrating apoptosis and glucose metabolism [52,73,74]. Also, interaction of HK-II with VDAC (voltage dependent anion channel), outer mitochondrial pore- forming protein supply cellular ATP by coupling OXPHOS and glycolysis [75]. The VDAC/HK-II interaction blocks apoptosis through multiple mechanisms [76], such as by disturbing the pore formation of mitochondrial permeability transition [77] and by proapoptotic protein inhibition, which targets outer mitochondrial membrane [78]. Several studies have shown the potential of HPV oncoproteins, E6/E7, to induce expression of HK-II [79]. It is interesting, since it provides a direct link between the HPV oncogenes and the expression of major cellular enzymes responsible for apoptotic resistance and metabolic reprogramming in cancer cells. This leads to the increased oncogeneicity and decreased therapeutic sensitivity in the clinic [40]. Certain studies have stated that PI3K/Akt pathway is utilised via the HK-VDAC disruption which ultimately results in apoptosis. However, glycolysis may produce an enhanced levels of lactic acid

and which facilitates the tumor cells to outbreak from the detection of the immune system and allow rapid proliferation [80]. It has been reported that HPV mediated stimulation of HK-II are dependent on the overexpression of E6/E7 oncoproteins [81,82]. Two major mechanisms are involved in HK-II stimulation by HPV. First, repression of HR-HPV16 results in downregulation of the MYC transcript [83] and protein levels, thereby repressing the transcription of HK-II [84]. Over-expression of HR-HPV-E6 in Mouse Embryonal Fibroblast (MEF) associated the upregulation of HK-II expression through Myc [79]. Furthermore, stabilization of c-Myc by HR-HPV-E6 increases its protein turnover. Second, the expression of miR-143-3p is significantly induced by E6/E7 repression, suggests that HK-II inhibitory miRNA is downregulated by the induction of E6/E7. Both the mechanisms result in HK-II stimulation by HR-HPV-E6 expression in HNSCC and cervical cancer cells [73]. Additionally, HR-HPV-E6 mediated p53 degradation regulates the transcription of HK-II by binding to its promoter region along with changes in the concentration of glucose, and oxygen [85]. c-Myc is a transcription factor which is reported to be a factor that interacts with HR-HPV-E6 and promotes the expression of glycolytic genes such as HK-II, LDH-A, GLUTs, enolase A and phosphofructokinase (PFK) [86,87]. In HPV^+ and HPV^- HNSCC, the enhanced glycolysis is promoted by the upregulation of HK-II, which acts as a precursor as well as provides energy for the growth of the tumor [88]. In HPV^+ HNSCC, Zeng Q et al., (2017) has reported the upregulation of HK-II, leading to enhanced glycolysis in a c-Myc-dependent manner when there was an ectopic over-expression of HPV16-E6/E7 in mouse embryo fibroblasts [89]. This suggests that the oncoproteins E6/E7 directly activates HK-II expression and is responsible for the reprogramming of HPV transformed cells to regulate glycolysis [81]. Along with this, in HPV^- HNSCC anaplerosis, HK-II plays a major role in carbon utilization derived from glutamine, thus, supplying intermediates for the Krebs cycle and facilitates the maintenance of homeostasis by inducing autophagy in response to glucose deprivation [75].

Lactate dehydrogenase A (LDH-A) enzyme is responsible for catalyzing the final step of anaerobic glycolysis by converting pyruvate to lactate. LDH-A is shown to be highly upregulated in HNSCC [37]. There are five known active isoenzymes of LDH present in a human. LDH-A and LDH-B encode 2 extensive subunits A and B. LDH isoenzymes efficiently catalyze pyruvate to lactate if there are more A chains than B. Conversely, excess of B chains gives the advantage to catalyze pyruvate to acetyl-CoA. Conversion of pyruvate into lactate leads to the reduction of NADH to NAD^+, which is again reused in the glycolytic cycle and drive the process of glycolysis in cancer cells. LDH-A is a major enzyme involved in maintaining the glycolytic phenotype in cancer cells [90]. Study shows that Myc oncogene regulates the expression of LDH-A [91]. Inhibition of LDH-A in HNSCC cells by siRNA has shown to reduce the growth of tumor [92]. This suggests that inhibiting LDH-A activity could be important and effective anti-tumor therapy [93]. The incidence of aggressive tumor behaviour could be explained by the accumulation of lactate even in the presence of oxygen (Warburg effect) by LDH-A [94]. This creates an intracellular acidic environment for cancer cells mediating secretion of metalloproteinases and hyaluronidase resulting in matrix degradation by tumor associated fibroblasts (TAF) [95,96]. Additionally, the accumulation of lactate by LDH-A inhibits the activity of T Cells and dendritic cells contributing to the immunologic escape for HNSCC cells and thus inducing angiogenesis [97,98]. The tumor suppressor genes p53 and pRb gets inactivated after coming in contact with HPV oncoproteins, E6 and E7, which successfully modulates the metabolic machinery in HNSCC [99]. HPV-E6 oncoprotein facilitates the activity of mammalian target of rapamycin (mTOR) signalling pathway [100]. The accumulation leads to the induction of Warburg phenomenon by enhancing the glycolysis rate and LDH-A elevation, and lactate production [68]. Also, HPV-E7 oncoprotein leads to the induction of pyruvate kinase M2 (PKM2) isoform by acetylation and therefore promotes conversion of pyruvate to lactate by LDH-A. Additionally, HR-HPV-E2 oncoprotein promotes the HPV genome integration into host cell genome, and interact with the mitochondrial membrane. This

results in the production of reactive oxygen species (ROS). The generation of ROS positively correlates with the production of lactate by LDH-A [101]. This observation suggests that HPV associated infection in HNSCC cells leads to an acidification in the tumor microenvironment. In HPV^- HNSCC, the conversion of pyruvate to lactate by LDH-A creates an intracellular acidic environment by the accumulation of lactic acid inside the tumor cells and thus activates transforming growth factor-β (TGF- β), vascular endothelial growth factor (VEGF), and interleukin (IL), [102] even under normoxic conditions. It was reported that in HPV^- HNSCC cells (SCC-25, UMSCC89); there was a significant upregulation of LDH-A as compared to HPV^+ HNSCC cells (UD-SCC-2, UPCI:SCC90) [103]. This suggests targeting these metabolic pathways could represent a promise therapeutic strategy for HPV associated HNSCC.

4.2. HPV in glutamine and TCA cycle

The tricarboxylic acid (TCA) also known as Krebs cycle is a central metabolic pathway responsible for redox balance and macromolecule synthesis. Various enzymes involved in Krebs cycle, such as isocitrate dehydrogenase (IDH), aconitase (aconitase hydratase), succinate dehyrogenase (SDH), fumarase (FH), α-ketoglutarate dehydrogenase complex (KGDHC) are deregulated and mutated in the majority of cancers including HNSCC [91,92]. Data suggest that targeting TCA can pave a new pathway to generate new therapeutic invention for treating cancer [104]. To meet the energy needs, cancer cells also utilize other fuel sources besides glycolysis such as glutamine to feed the TCA cycle [105, 106].

In HPV^- HNSCC, apart from glucose, another major source essentially playing role to fuel TCA cycle is amino acids [107]. Amino acids before entering into the TCA cycle is converted into the intermediates of α-keto acid: either oxaloacetate, pyruvate, acetyl-CoA or succinyl-CoA. The amino acid found in abundance in the human body is glutamine, which plays a major role in nitrogen transport in plasma membrane for biosynthesis of fatty acids and non-essential amino

acids, such as purines and pyrimidines, or entering the TCA cycle in the form of α-ketoglutarate (α-KG) [108]. The process of glutaminolysis i.e. the conversion of glutamine by glutaminase (GLS) to glutamate complements provides high energy cofactors [109]. Due to upregulated glycolysis and conversion of glucose to lactate in head and neck cancer, HNSCC cells (HN30 and HN31) utilize anaplerotic reactions to replenish the intermediates of TCA cycle via glutaminolysis. For doing so, cancer cells are responsible for upregulating both glutamine transporters as well enzyme catalyzing glutaminolysis [110]. HR-HPV-E6 oncoprotein degrades the p53 through proteasomal degradation [111]. p53 is a tumor suppressor which regulates cellular metabolism via the regulation of glycolysis and OXPHOS by the transcriptional activation of its downstream gene, *Tp53*-induced glycolysis and apoptotic regulator (TIGAR) [65]. p53 also activates the expression of proteins, such as the oxidase 2 (COX2), cytochrome c, ferredoxin reductase (FDXR) and apoptosis-inducing factor (AIF), which facilitate mitochondrial integrity [112,113]. p53 activates OXPHOS in cancer cells via the inactivation of pyruvate dehydrogenase kinase 2 (PDK2). PDK2 is an enzyme responsible for the inhibition of pyruvate dehydrogenase complex (PDC). The PDK inhibition in cancer cells, induces the production of acetyl-CoA via PDC, an essential molecule in Krebs cycle [114]. p53 has been reported to play a critical role in glutamine metabolism pathway, an alternate path that feeds the Krebs cycle. Thus, p53 degradation by the HR-HPV-E6 oncoprotein plays a critical role in reprogramming tumor cell metabolism [115].

4.3. HPV and oxidative phosphorylation

The powerhouse of the cell, mitochondria is the centre for generating ATP [113]. Mitochondria plays important role in cell death and signalling through regulating multiple processes like OXPHOS, β-Oxidation of fatty acid and other aspects such as biogenesis, fusion and fission [116]. Therefore, the involvement of mitochondria is critical in cancer which helps the cells to adjust themselves according to their metabolic requirements. In addition, mitochondria play a major role in the treatment of cancers. It has been reported that HPV^+ HNSCC cells (UP90 and UP154) favour

mitochondrial respiration over glucose metabolism, because of the elevated levels of cytochrome c oxidase (COX), a respiratory related enzyme and the high ratio of COX/HKII (Fig.5) [10]. However, HPV^- and p53 mutated HNSCC cells favour glycolysis as compared to OXPHOS for their survival [79]. The HR-HPV-E2 oncoprotein is a negative regulator of E6 and E7 [117]. Whereas, the E2 protein aggressively moves between the nucleus and cytoplasm. The position of E2 protein majorly determines the incorporation of HPV genome to the host. As reported earlier, E2 protein can induce apoptosis in cytoplasm and DNA breaks and chromosomal instability in nucleus [118]. It was reported that mitochondrial membrane also contains E2, which changes the cristae morphology and enables elevated release of ROS, which leads to the modification in cellular respiration [119]. Mitochondrial ROS production is majorly mediated by proteins from complex III and ATP synthase, which regulates the cristae structure. Consequently, E2 protein could modulate the mitochondrial function and ROS release via the interaction with these proteins [119]. It has been reported that co-expression of E2 and E1 proteins negatively regulates of glutathione levels and superoxide dismutase levels through increasing the ROS levels in tumor cells [120]. Recently, the upregulation of CAIX in HPV^- as compared to HPV^+ also reported in HNSCC cells. CAIX has also been shown to reduce cell adhesion mediated through E-cadherin mediated-β- catenin axis in HNSCC [121].

5. mTOR, Akt and Myc regulation in HPV infected HNSCC

The HPV-E6 oncoprotein is responsible for promoting high metabolic phenotype through increasing the activity of mTOR [122]. mTOR is highly upregulated and associated with metabolic dysregulation in HNSCC (Fig.5). mTOR increases the expression of PKM2, PDK1, HIF-1α, LDH and GLUT1 [74]. These pathways play important role in altering the function of cellular metabolism, which is directly mediated by HR-HPV-E6 & E7. It has been demonstrated in many studies that mTOR signalling is activated/upregulated in >80% of HNSCC. Over activation of mTOR results in the increased glucose uptake and uncontrolled glycolysis regulation in HNSCC,

which results in the increase in lactic acid production [108,109]. The expression of HR-HPV-E6 & E7 in HPV infected cells promotes the destabilization and degradation of p53 and pRb which leads to the initiation and propagation of cancer. With the help of signalling pathways such as PI3K/Akt/mTOR, these oncoproteins alter various cellular and molecular events [32]. The radiation sensitivity of HPV^+ HNSCC also correlates with Akt activation [123]. Studies have shown that the mTOR inhibitor CCI-779 plays a key role in inhibiting proliferation of HNSCC cells and tumorigenicity when combined with external radiation therapy (XRT) as well as chemotherapeutic agents. The transcriptional activity of p53 is linked with overexpression of Myc and increased expression of HK-II, which is closely linked with enhancing glutaminolytic flux via glutamate dehydrogenase [124].

In HNSCC, p53 also dysregulate the glycolysis and energy supply through GLUT1 and GLUT4 [65]. It has been reported that p53 can also suppress the transcription and expression of GLUT3 through blocking NF-κB activation [125]. TIGAR is a downstream target of p53 and have an enzymatic activity similar to the bi-functional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2/FBPase-2), which is responsible for the degradation of fructose-2,6-bisphosphate (Fru-2,6-P2) [126]. Fru-2,6-P2 allosterically activates PFK1, rate limiting enzyme in glycolysis. Therefore, p53 leads to the downregulation of Fru-2,6-P2 via TIGAR, thereby deactivates PFK1 and inhibits the flow of glycolysis in HNSCC cells. Additionally, p53 plays a major role in PPP by inhibiting it in a glucose 6-phosphate dehydrogenase (G6PD)-dependent manner [127]. PPP is correlated with the biosynthesis of nucleotides through involving p53 in restricting the tumor growth by limiting the nucleotides supply required for DNA replication during cell division. Whereas, the mutant p53 have been reported to play an important role in the upregulation of HIF-1α, resulting in the increased glycolysis, fibrosis, angiogenesis contributing to cancer progression (Fig. 6) [128].

The transcription network of c-Myc is linked to majority of biological processes such as cellular growth and proliferation, development, cell cycle, apoptosis and energy metabolism. It has been reported that HPV-E6 and Myc binds to the DNA and modulates cell proliferation and cell differentiation through activating the telomerase reverse transcriptase (Fig.5) [86]. In majority of cancers, including HNSCC, the frequent dysregulation of Myc alters cellular metabolism and induce new blood vessels formation through HIF-1 signalling [84]. The specific markers responsible for alteration in HNSCC such as HK-II, PDK1, LDH and VEGF are targets of HIF-1 and are regulated by c-Myc [117, 118]. Myc is also responsible for transcriptionally activating the key enzyme regulating glutaminolysis is and TCA cycle. Myc promotes glutamine import into the cells through upregulating glutamine transporters, such as ASC amino acid transporter 2 (ASCT2) and system N transporter (SN2) [129]. Myc also converts glutamine to glutamate through activating glutaminase (GLS1) via transcriptional suppression of its negative regulator miR23a/b [130]. It plays major role in oxidation of fatty acids and metabolites into the TCA cycle through overexpression of fatty acid transporters such as fatty acid-binding protein 4 and hydroxyacyl-CoA dehydrogenase [131].

6. Impact of the oral microbiome on oral cancer

The oral microbiota plays crucial role in the human health. It maintains and controls the balances between host and microbes in oral cavity. Disbalances in the association between microbes and host in oral cavity leads to the development of oral disease. This is generally caused by bacteria and viruses that can eventually lead to the development of cancer [132]. Combination of smoke, viral infection (HPV), poor oral hygeine, alcohol consumption enhances the risk of OSCC. Research shows tobacco consumption and viral infection modulates the oral microbiota profile. Stewart et al., 2018 showed the effect of tobacco consumption and use of electronic ciggarettes (EC) on the oral microbiome. It was observed that use of EC had no certain effects on the oral microbiome. However, consumption of tobacco and constant smoking had a significant effect on the resident

bacterial population on the oral microbiome. Also, tobacco consumption resulted changes in the sputum microbiota [133]. In another study, Sharma et al., 2020 identified DNA damage in the oral cavity at much higher rates in persons with high tobacco consumption. The smoke in tobacco results in exposure to carcinogens and toxic constituents, and further development of malignancies such as oral cancer. The tobacco specific two carcinogens, nitrosamine 4-(methylnitrosamino)-1(-3-pyridyl)-1-butanone (NNK) and N-nitrosonornicotine (NNN) have been shown to trigger alterations that interfer with the replication, transcription, and DNA repair machinery in the oral microbiota. The altered oral microbiome due to tobacco consumption can cause disruption of host cell defense mechanisms, causing chronic inflammatory conditions resulting in a cascade of changes causing DNA damage [134]. Smoking can also result in disruption in the structure of microbial biofilm in the oral microbiome and can cause unstable colonization, rising the susceptibility to bacterial infections in smokers by modulation of innate and adaptive immunity. Additionally, smoking can result in disruption of commensal niches. Furthermore, studies have revealed the involvement of *Streptococcus spp*. as a cofactor in the transformation of oral keratinocytes by HPV resulting in malignancy. The infection of Mycoplasma also enhances the HPV infection rate in the oral microbiota. This resulted in modulation of glutamate metabolism, and metal transport triggering the infection [135]. However, the effect of the virus and its potential role in the metabolic activation and alteration of oral microbiome is still unclear and needs further investigation. The chronic infection constitutes the major factors responsible for cancer pathogenesis, manifesting the resident microbiota which regulate oral surrounding homeostasis [136-138]. The changes in oral microbiota modulate the association between human diseases and oral microflora. Oral microbiota seems to modulate oral carcinogenesis via modulation of cell metabolism, for example modifying the vitamins and nutrients constituents, thereby increasing the production of various cytokines known to play a critical role in various pathological conditions [139]. The upregulation of several inflammatory markers and cytokines result in the alteration of various metabolic pathways which

ultimately result in cancer progression. For example, the protein expression of RAGE changes significantly after peridontal infection caused by oral microbiota which leads to carcinogenesis [140]. Additionally, viruses such as HPV and gram negative bacteria, secrete IL-6, TNF-a by binding to leucocytes TLR receptors. These inflammatory markers supports tumor spread by creating a pro-angiogenic environment [141]. In the oral microbiome, the chronic infection caused by virus or bacteria result in metabolic changes such as sulfur compounds, free radicals, and acid generation [142]. Also, certain bacteria can metabolize alcohol to acetaldehyde that leads to genotoxicity and consequently neoplastic transformation. Furthermore, L-tryptophan metabolism in the oral microbiome to secondary metabolites such as pyruvate, indole have been shown to be associated with cancer progression. Another mechanism by which microbiota modulates the host miRNAs expression is by the production of various metabolites that results in significant changes in the metabolism of host-cell. This results in gene alteration and changes in the miRNA expressions which favors again the OSCC progression [143-145].

6. Therapeutic approach for targeting HPV$^+$ and HPV$^-$ HNSCC

Metabolic alteration in solid tumor is considered as a potential therapeutic target. Since cancer cells have an enhanced rate of proliferation, they follow the Warburg phenomenon to compensate their need for energy. Blocking the Warburg phenomenon by one or the other mechanism leads to the death of cancer cells selectively [144]. Additionally, it has also been demonstrated that the majority of cancer cell has functional mitochondria irrespective of the earlier known evidence that the mitochondrial activity is disrupted in cancer cells [22]. Glycolysis as well as functional mitochondria facilitate tumorigenesis in cancer cells. Earlier, it has been demonstrated that there is an explicit difference between HPV$^+$ and HPV$^-$ HNSCC. Also, it has been shown that the prognosis rate of HPV$^+$ HNSCC is better as compared to HPV$^-$ in response to the radiation treatment [145]. This clear difference facilitates the recognition of HPV$^+$ and HPV$^-$ HNSCC as two separate diseases. Despite the fact, both HPV$^+$ and HPV$^-$ HNSCC are treated in a similar manner. HPV$^-$

tumors have shown to be highly glycolytic than the HPV^+, making it clinically more helpful to find novel targets for the HPV based subtypes of HNSCC. A major issue during the treatment process is the occurrence of toxicity. Therefore, gradually, nowadays research is focusing on developing new and innovative therapeutic strategies to improve the outcomes especially focusing on the cases of HPV^+ patients and with the aim of raising standard therapeutic strategies for HPV^- patients. Irrespective of enormous studies, there is still no effective cure for HPV related diseases. There are three vaccines available against HPV: Gardasil, Gardasil 9 and Cervarix [146], which can be used to prevent HPV associated cancers. Several components and drugs have been analyzed against the E1 and E2 viral proteins that are necessary for HPV genome replication (Table 1) [147].

In addition, targeting the viral genome is one other alternative effective approach towards the HPV infected treatments. One such compound belongs to the pyrrole-imidazole, which binds to the DNA Sequence specifically and targets the viral genome [148]. These compounds bind to AT-rich regions in the origin of HPV replication region where E1 and E2 binding sites are located, this affects the stability of episomal viral genomes. Also, antibody for epidermal growth factor receptor (EGFR) and radiation is considered as an alternative for HPV^+ HNSCC [149]. Axalimogene filolisbac (AXAL or ADXS11-001) is a novel immunotherapeutic based drug which is currently being used against HPV associated HNSCC [150,151]. The use of inhibitors targeting mitochondria is a possible consideration to treat HPV^+ HNSCC metabolism. Phenformin/metmorphin, for example besides activation of the PI3K pathway, inhibits Complex I of electron transport chain (ETC) and Krebs cycle intermediate in HNSCC cells [152]. Christopher T. Lucido et. al., (2018) demonstrated the expression of β2AR that was correlated with the enhanced mitochondrial metabolism in HPV^+ HNSCC as compared to HPV^- HNSCC. Targeting β2AR with β-blockers, such as propranolol has been shown to inhibit primary tumor growth in HPV^+ HNSCC cells [153,154]. Also, VLX600 has been shown to reduce mitochondrial metabolism in tumor cells, which along with DCA (glycolytic inhibitor) has been proven as an effective therapy against HPV^+

HNSCC metabolism through targeting mTOR signaling [155]. On the other hand, since glycolysis is enhanced significantly in HPV^- HNSCC as compared to HPV^+, anti-glycolytic drugs are considered as the best way for chemotherapy as to enhance the antitumor response rate. In 2008, Simons et al., reported the cisplatin cytotoxity in HNSCC xenografts, which is mediated by 2-Deoxy-D-glucose (2-DG) [156]. Additionally, 3-bromopyruvate (3-BP) and lonidamine are the most commonly tested inhibitors against HK-II and are used in both pre-clinical as well as clinical model either in combination or alone with radiation therapy and chemotherapy [157]. 3-BP is responsible for inhibiting glycolysis by disrupting the connection between the outer membrane of mitochondria and HK-II, causes the deactivation of HK-II. It has been demonstrated that HK-II inhibition by using siRNA and a targeted HKII-VDAC complex induces apoptosis in cancer cells [158]. Tyrosine kinase (TK) inhibitor, for example imatinib decreases glycolysis, hampers various glycolytic enzymes such as Hexokinase and Phosphofructokinase 1 via HIF-1 [114]. Recently, Zhang et al., 2017 reported the role of miR-143 in oral cancer and identified HK-II as a direct target of miR- 143 in patients' tumor and oral cancer cells, suggesting that inhibiting HK-II by miR-143 might be a therapeutic approach for treating oral cancer (Table 1) [159].

Combinatorial effect of 2-DG with 6- aminonicotinamide, a PPP inhibitor results in increased cytotoxic effect, which proposed a new theory of targeting multiple metabolic pathways which could effectively treat the disease cancer [160]. This suggests that glycolysis via PPP plays an important role in contributing the tumorigenicity in HNSCC. AZD3965, most effective MCT1 inhibitor is currently under clinical trial phase I and has been shown to decrease the growth of the tumor by inhibiting the release of lactate and has been an effective drug for HPV^+ HNSCC. Cetuximab inhibits proliferation of HPV^- HNSCC cells through inhibiting glycolysis via LDH-A by downregulation of HIF-1α, therefore reversing the Warburg phenomenon which is critically important for the survival and proliferation of cancer cells [161] Inhibitors, such as orlistat, blocks

the synthesis of fatty acids via fatty acid synthase which might be another effective approach to enhance the treatment response towards HPV associated HNSCC (Table 1) [162].

Tumor cell metabolism is being highly studied as a novel area for new biomarkers and assumed to be key targets for novel pharmacologic interventions. New metabolic targets continue to be identified in different types of tumor. Altogether, there is convincing evidence that HNSCC requires elevated rate of glucose uptake and conversion for cell survival and progression. This appears to make HNSCC susceptible to targeted therapies employing inhibitors to glycolytic genes or enzymes. Even the understanding of increased glycolysis mediating secondary energetic pathways such as glutaminolysis, Pentose Phosphate Pathway, serine pathway provides new targets to be inhibited for disease treatment. Identifying the key factors involved in oncogenic events and behind altered HNSCC metabolism may have important therapeutic implications.

7. Conclusions and prospective

Cancer cell metabolism plays important role in survival and progression with the help of major metabolic pathways that produce energy. So, this process of metabolic reprogramming has made its way into the hallmark of representing the disease. In head and neck cancer, HPV plays critical role in modulating their metabolism, thereby facilitating the tumor progression. The HPV oncoproteins, E6 and E7, regulate several enzymes, which are involved in metabolic pathways in HNSCC. The altered metabolism in HNSCC, provides sufficient ATP through glycolysis, which helps in meeting the high-energy demands of HNSCC during cell proliferation, migration and invasion. Additionally, the E6/E7 oncoproteins, alter several signalling pathways including mTOR, Akt, c-Myc, HIF-1 etc., which in turn alter the metabolic phenotype in HNSCC. Although, there are invasive treatments available such as larger excision procedures, cryotherapy, electrosurgery, laser therapy etc. but not all HPV+ HNSCC respond properly to the treatment apart from it they acquire the ability to resist such chemotherapeutic strategies. This suggests that there is an urgent need of development of new

strategies to overcome these issues. Therefore, focussing on cancer cell metabolism associated with HPV status could effectively bring out new therapeutic development which could be helpful to manage the disease.

Conflict of interest statement

The authors declare there is no conflict of interest.

Acknowledgements

We thank our lab members and collaborators for carefully reading the manuscript and contributing valuable inputs for improving the manuscript. S.R. and D.K. were supported by the Department of Science & Technology- Science and Engineering Research Board (DST-SERB) funded research grant (ECR/2016/001489), Govt. of India.

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Figure Legends

Figure 1. Factors associated with HNSCC. Consumption of tobacco products and alcohol are the major risk factors involved in HPV^- HNSCC. Whereas, HPV infection mediates HPV^+ HNSCC. The HPV^- HNSCC patients of younger age have a poor clinical outcome as compared to HPV^+ HNSCC, whereas the HPV^+ HNSCC have an improved clinical outcome. The anatomical location of HPV^- HNSCC involve all head and neck cancer sites, while in HPV^+ HNSCC, the oropharyngeal region is the major site of infection. Mutation in p53 and pRb are the major reason behind HPV^- HNSCC, while E6 mediated inactivation of p53 and E7 mediated degradation of pRb is the major reason behind HPV^+ HNSCC.

Figure 2. In normal cells, the glycolysis and OXPHOS are regulated in a synchronized manner. Glusose is converted to pyruvate in aerobic conditions and generates 2 ATP through glycolysis. Pyruvate is then converted to acetyl CoA and regulated in OXPHOS for the generation of total 36 ATPs. Under anaerobic condition, pyruvate is converted to lactate by LDH-A for the generation of ATP. In HNSCC cells, solid tumors favor glycolysis as compared to OXPHOS for the generation of ATP even in the presence of oxygen following Warburg phenomena. HNSCC cells shift the metabolic flow from OXPHOS to glycolysis. OXPHOS (Oxidative phosphorylation); ATP (Adenosine triphosphate); LDH (Lactate dehydrogenase).

Figure 3. In HPV^+ Hypoxia, VHL mutation and stimulation of growth factor such as PI3K/Akt/mTOR, MAPK, EGFR causes accumulation and stabilization of HIF-1. Under hypoxia HIF1α and HIF-1β binds to the HRE in the DNA segment promotes the transcription of genes involved in metabolism (GLUT, HK, LDH, GADPH), regulation of pH, angiogenesis (VEGF, TGFβ), proliferation(TGF α, Cyclin G2), apoptosis(BNIP3, NIX). VHL(von Hippel-Lindau); GLUT(Glucose transporter); HK(Hexokinase); LDH(Lactate dehydrogenase); G6PD (Glucose-6-phosphate dehydrogenase), VEGF (Vascular endothelial growth factor); TGF(Transforming growth factor); TGF(Transforming growth factor); BNIP3(BCL2 Interacting Protein 3).

Figure 4. HR-HPV-E6 mediated degradation of p53, upregulates the expression of GLUT1

and elevates the uptake of glucose uptake in HNSCC. SGLT Na^+ve /glucose co-transporters through Na^+ve /K^+ve ATPase secondary active transport causes glucose internalization in epithelial cells. SGLT1 $Na^{+ve/}$glucose co-transporters present in HPV^+ HNSCC facilitates cells to accumulate glucose even in the presence of low concentrations of glucose. Highest accumulation of FDG can be detected using PET SCAN and resulting in poor patient survival.

Figure 5. HPV infection promotes glycolysis in head and neck squamous cell carcinoma. The E6 oncoproteins inhibits p53 by proteolytic degradation further activating HIF-1 which activates the glycolysis in head and neck squamous cell carcinoma. Akt/mTOR activation by E6 oncoprotein induces the high glucose metabolism promoting the cancer survival. E7 oncoprotein of the HPV activates the PKM-2 gene increasing the lactate production in the cancer cells. HPV presence, activates COX augments, upregulating ETC.

Figure 6. TIGAR, downstream target of p53 is a bi-functional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2/FBPase-2), responsible for fructose-2,6-bisphosphate (Fru-2,6-P2) degradation. Fru-2,6-P2 allosterically activates PFK1 causing p53 mediated downregulation of Fru-2,6-P2 via TIGAR, thereby inhibiting the flow of glycolysis in HNSCC cells. p53 plays a major role in PPP by glucose 6-phosphate dehydrogenase (G6PD) dependent manner inhibiton. p53 have been reported to play an important role in the upregulation of HIF-1α, resulting in the increased glycolysis, fibrosis, angiogenesis contributing to cancer progression

HPV$^+$		
Drug	**Target**	**NCT No.**
Gardasil	HPV 6, 11, 16, 18	NCT00092534
Gardasil 9	16, 18, 31, 33, 45, 52, and 58;	NCT03943875
Pyrole-imidazole	A-T regions of viral genome	-
Cetuximab	EGFR receptor	NCT01084083
Axalimogene	Immune-based therapy	NCT02540928
Propranolol	Pyruvate dehydrogenase Kinase	NCT02013492
VLX600	Electron transport chain (ETC)	-
Cervarix	HPV 16, 18	NCT00316693
HPV$^-$		
Silybin	GLUT	NCT03440164
Lonidamide	HK	NCT00435448
2-Deoxyglucose	G6P isomerase	NCT00633087
TLN-232	PKM2 dimers	NCT00735332
Dichloroacetate	PDK	NCT01111097
AZD-3965	MCT1	NCT01791595
CPI-613	Pyruvate Dehydrogenase	NCT03699319
Gossypol	LDH-A	NCT00540722
Galloflavin	LDH-A	Pre-Clinical Studies
Daunorubicin	GLUT-1	NCT02914977
Gefitinib	Tyrosine Kinase	NCT00049543
Erlotinib	Tyrosine Kinase	NCT02013206

Table 1: Current therapeutic strategies for HPV$^+$ and HPV$^-$ HNSCC