

Molecular genetics of head and neck cancer (Review)

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Abstract. Understanding of molecular regulatory pathways presents a novel therapeutic approach for the treatment of head and neck cancer. These specific check points are becoming the targeted therapeutic approach. In this review, we highlight certain major signaling mechanisms, which are involved in the pathophysiology of head and neck cancer. Also, we discuss the current ongoing trials based on the *in vitro* success of targeted therapies.

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1. Introduction

Head and neck cancer is the sixth most common type of cancer worldwide, with approximately 45,000 new cases occurring each year in the United States (1). Tobacco, alcohol and high-risk human papillomavirus (HPV) types 16 and 18 are considered to be associated risk factors for the development of head and neck cancer, acting as potent carcinogens leading to malignant transformation (2). In the process of oncogenesis, alterations in tumor suppressor genes and activation of oncogenes, which initiate various regulatory mechanisms, lead to malignant proliferation (2). In this review, we examine the key molecular pathways and gene-receptor interactions involved in the pathogenesis of head and neck cancer.

2. p16 and p53

It has been established that the proto-oncogene, *cyclin D1*, is amplified in head and neck squamous cell carcinoma (HNSCC) as well as in other malignancies (3), and one of the most common deleted gene loci in head and neck cancers occurs at chromosome 9p21-22 (4). This critical deletion includes the p16 region, a tumor suppressor gene, which suppresses *cyclin D1* (5). Therefore, any deletion or mutation occurring on p16 leads to *cyclin D1* amplification, which is most frequently encountered in various HNSCCs (5). Homozygous deletions or methylation in the 5' CpG region of p16, which causes the complete inactivation of p16 through the blockage of transcription, have been identified in approximately one-fourth of primary head and neck cancers (6).

Mutations in the tumor suppressor gene, p53, located on chromosome 17q13, are observed in approximately 50% of head and neck cancers (7,8). These mutations result in elevated dysfunctional p53 levels, a phenomenon secondary to loss of negative regulation via Mdm2 (murine double minute oncogene 2), a finding noted in tobacco users (9). In a study conducted by Field *et al*, heavy smokers showed elevated dysfunctional p53 ($p < 0.005$) when evaluated for squamous cell carcinoma of head and neck (10). The loss of function of p53 leads to uncontrolled growth, making cells incapable of appropriately responding to DNA damage or stress (11). Moreover, tobacco usage has been demonstrated to cause *Ras* (*Rat sarcoma*) mutations, as identified by studies in India and Taiwan, who have an enormous population of tobacco chewers (12). Of the *Ras* mutations, H-*Ras* has been implicated in HNSCC and is associated with tobacco chewing (12). Notably, mutations of H-*Ras* have been proven to be favorable in terms of prognosis, while low levels of H-*Ras* expression result in poor prognosis (12).

In HPV-16-related malignancy, the E6 protein is known to inactivate the p53 gene via ubiquitination, disturbing the cell cycle and DNA repair mechanisms (11).

Microsatellite instability is another key factor contributing to carcinomas, which is caused by mutations in the genome affecting DNA repair and replication (13). Field *et al* identified that non-smoker head and neck cancer patients had microsatellite instability (13). Upon studying 34 satellite markers, instability was observed in 28% of head and neck cancers by at least 2 or more markers. Furthermore, microsatellite instability and loss of heterozygosity are of particular significance

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in specific regions located on chromosome 3 and 17, which are highly relevant molecular targets for carcinogenesis (13,14).

3. Regulatory pathways

The insulin-like growth factor, produced by the liver, is growth hormone-dependent. Insulin-like growth factor-1 receptor (IGF-1R) is a transmembrane receptor composed of 2 α and 2 β subunits, which possesses tyrosine kinase activity (15). Its usual functions include affecting cell growth, protecting cells from apoptosis, and influencing focal adhesion stability, cell-cell contact and cell motility (2). It is expressed in a number of tumors, including HNSCCs (11). As p53 inhibits the activity of IGF-1R, any mutations of p53, as observed in HNSCCs, result in the increased expression of IGF-1R (15). Additionally, a number of studies have linked the excessive expression of IGF-1R to metastatic and invasion capabilities (2). Through signal transduction, the downstream phosphorylation of the insulin receptor substrate-1 (IRS-1) occurs, which in turn stimulates the mitogen-activated protein kinases (MAPKs) and the phosphoinositol 3 kinase/protein kinase B (PI3-K/Akt) pathway (16).

PI3-K/Akt is a downstream regulatory pathway, which is activated by the tyrosine kinase receptor family (2). When Akt, the central signaling molecule for PI3-K, is activated, it augments the expression of several proliferative and anti-apoptotic proteins, such as Bcl-2, Bcl-x and NF- κ B (2). Furthermore, Akt phosphorylates various targets in the cytoplasm in conjunction with chaperone proteins (17). It also serves to increase glycogen synthesis and cell metabolism via the forkhead family of transcription factors (FOXO) and glycogen synthase kinase 3 (GSK3) inactivation (18). Other causes of Akt activation include the direct mutation or amplification of PI3-K, activation of the Ras oncogenes and diminished expression of the phosphatase and tensin homolog (PTEN), which is a known inhibitor of the PI3-K/Akt pathway (19). Studies have indicated that the loss of PTEN expression along with the activation of Akt correlates with poor prognosis of squamous cell carcinoma of the tongue (2).

The mammalian target of rapamycin (mTOR) is a downstream regulatory pathway associated with PI3-K/Akt (2). mTOR is a serine/threonine kinase, which is required by PI3-K/Akt to exert its full effect (2). Upon activation via Akt, mTOR phosphorylates the p70-S6 kinase, a translation regulator, which then activates the ribosomal protein S6, enhancing the control of cell growth through increased mRNA translation (2). The eukaryotic translation initiation factor 4E (eIF 4E) also plays a vital role in translation and hastens protein synthesis, while preparing for cell division (20). Rapamycin, a known inhibitor of mTOR, is involved in inducing apoptosis and inhibiting the growth of HNSCC cells. mTOR inhibitors (e.g., rapamycin and temsirolimus) are currently being evaluated in the treatment of head and neck cancers (2). Amornphimoltham *et al* demonstrated that the S6 ribosomal protein in HNSCC cell lines was decreased upon treatment with rapamycin, thus affecting the translational activity of the cell cycle (21). In a study conducted by Ekshyyan *et al*, the effect of 3 weekly doses of 25 mg of temsirolimus on the Akt/mTOR pathway biomarkers was evaluated in tumor and peripheral blood mononuclear cells (PBMCs) of patients

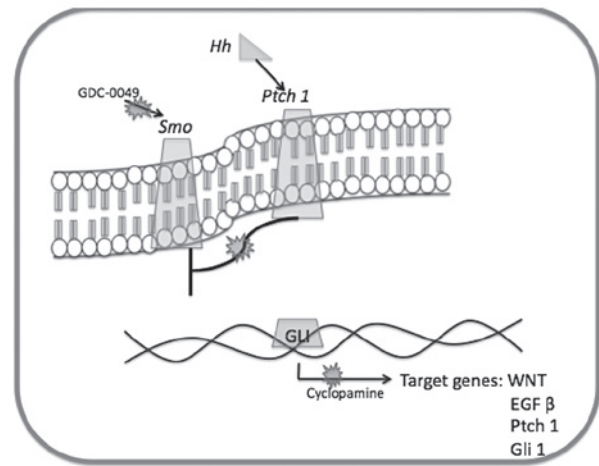


Figure 1. Hedgehog signaling pathway. As the Hh protein binds to the Ptc1 receptor, the normal inhibition on Smo is released. Consequently, there is activation of Gli (glioma-associated transcription factors) and expression of the target genes. Hh, Hedgehog; Ptc1, patched 1; Smo, smoothened; Gli, glioma-associated oncogene homolog 1; EGF, epidermal growth factor receptor.

with HNSCC. They noted a significant inhibition of the mTOR pathway in tumors and PBMCs of HNSCC with minimal side-effects (22).

Hedgehog (Hh), a secreted protein, is involved in a number of embryonic developmental processes and maintaining stem and progenitor cells (23). The Hh signaling pathway is composed of 2 receptor complexes, patched (Ptc) and smoothened (Smo) (23). Once the Hh protein binds to the Ptc receptor, the normal inhibition on Smo is released, thus activating the pathway (24). This leads to the activation of the transcription factors, the glioma-associated oncogenes (Gli1, Gli2 and Gli3), and the expression of target genes [WNT, transforming growth factor (TGF) β , Ptc1 and Gli1] (24). Furthermore, there is an upregulation of the apoptotic inhibitor, Bcl-2, the cellular caspase-8-like inhibitory protein (cFlip) and the platelet-derived growth factor α (PDGF α) (24). Hh signaling is implicated in the development of sporadic basal cell carcinoma and continues to be a target of investigation for enhancing treatment modalities (25). Cyclopamine, an Hh signaling inhibitor, has been used in trials for the treatment of basal cell carcinoma, specifically inhibiting Hh target genes (Fig. 1) (25). Yan *et al* investigated the significant therapeutic potential of cyclopamine in oral squamous cell carcinoma, in which 44% of enrolled patients expressed Gli (26). They identified that cyclopamine effectively inhibited Gli expression, causing cell cycle G1 arrest, slowing of cell growth and inducing apoptosis (26). GDC-0449 (Vismodegib) acts at the level of Smo receptor inhibition in targeting basal cell carcinoma, and is currently being studied in clinical trials as a potential anti-tumor agent (27).

The epidermal growth factor receptor (EGFR) signaling pathway is of high significance in head and neck cancer pathology (2). EGFR, a tyrosine kinase receptor expressed on epithelial cells, is overexpressed in HNSCC (28). However, EGFR expression rates are lower in laryngeal tumors versus squamous cell carcinoma of the oral cavity or oropharynx (2). Several autocrine ligands, including TGF α and amphiregulin, activate the EGFR receptor, forming a dimer, which causes

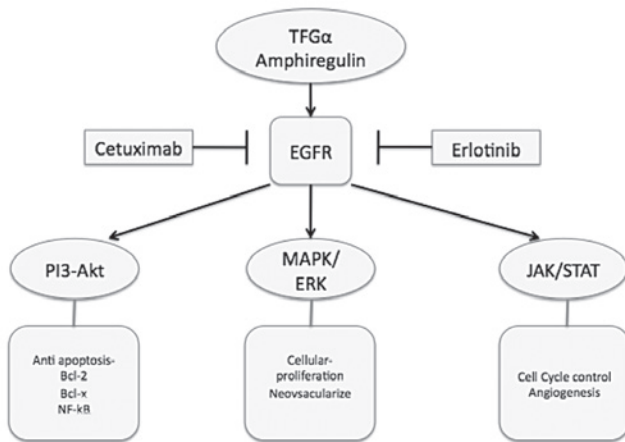


Figure 2. EGFR pathway. Upon activation, EGFR activates a number of pathways which produce cellular effects. EGFR inhibitors, including cetuximab and erlotinib, particularly produce anti-tumor-like activity. EGFR, epidermal growth factor receptor; TGF α , transforming growth factor α ; JAK/STAT, Janus kinase/signal transducers and activators of transcription; PI3-Akt, phosphoinositol 3 kinase/protein kinase B.

ATP-dependent autophosphorylation of the intracellular tyrosine residues (23). Upon phosphorylation, EGFR can trigger several downstream pathways, including PI3-K/Akt, sending anti-apoptotic signals, and MAPK/ERK, regulating cell proliferation, tumor induced neovascularization, invasion and metastasis (2). The Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway is also activated conferring additive effects of cell cycle control and angiogenesis to the pathway (2). Evidently, there is overlapping of the downstream pathways between EGFR and IGF-R1, providing a probable advantage for using anti-tyrosine-kinase receptor therapies which combine the two receptors (2). Current targeted therapies include the use of monoclonal antibodies (e.g., cetuximab), highly specific for the EGFR receptor and tyrosine kinase inhibitors (TKIs; e.g., gefitinib and erlotinib) (28). TKIs have demonstrated respectable improvement in overall and progression-free survival (28). Treatment with erlotinib in HNSCC improved progression-free survival for approximately 9.6 weeks, and 3.3 months upon treatment with erlotinib and cisplatin combination therapy (28). Therefore, it has been suggested that the TKI effect on survival is quite comparable with standard chemotherapeutic agents in the treatment of HNSCC (28). Cetuximab, an IgG1 monoclonal antibody, has an antagonistic effect on the EGFR receptor, hindering downstream signaling and mediating the natural killer cell-dependent lysis of HNSCC cells (Fig. 2) (28-30). When used with adjuvant radiotherapy, cetuximab has been demonstrated to be particularly efficacious, demonstrating an overall survival improvement of 29.3-49.0 months and a progression-free survival of 3.3-5.6 months (28).

4. Future research

Better understanding of the molecular pathogenesis in HNSCC is the key to developing targeted therapies. It is clear with our increased understanding of the tumor biology that agents targeting multiple pathways will be more successful owing to the complex signaling in HNSCCs. As demonstrated

in this review, molecular pathogenesis and genetic associations are the key to future therapies. In an ongoing phase II trial, temsirolimus with addition of cetuximab is under evaluation in patients presenting with recurrent or metastatic head and neck cancer (31). Also, in a similar population, the effects on survival rates of using cetuximab with bevacizumab, an inhibitor of angiogenesis, compared with single agent treatment are currently under investigation in phase II trials (32). GDC-0449 is currently being investigated further in regards to its effect on response and survival rates in the treatment of basal cell carcinoma (33). Despite recent advances, survival has remained relatively unchanged over the past few decades underscoring the need for more effective therapeutics.

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