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Review

# Genomic sequencing and precision medicine in head and neck cancers

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**Abstract**

Head and neck squamous cell carcinoma (HNSCC) remains a common and deadly disease. Historically, surgical and chemoradiation treatments have been met with modest success, and understanding of genetic drivers of HNSCC has been limited. With recent next generation sequencing studies focused on HNSCC, we are beginning to understand the genetic landscape of HNSCCs and are starting to identify and advance targeted options for patients. In this review, we describe current knowledge and recent advances in sequencing studies of HNSCC, discuss current limitations and future directions for further genomic analysis, and highlight the translational advances being undertaken to treat this important disease.


*Keywords:* Head and neck cancer; HNSCC; Genomics; Precision medicine; Personalized medicine

## Introduction

Head and neck squamous cell carcinoma (HNSCC) remains a common and highly aggressive disease. It is the sixth most common cancer worldwide; in 2012 alone there were over 600,000 new cases and over 375,000 deaths attributed to HNSCC.[1] While tobacco and alcohol historically have been the most important etiologic factors in the development of HNSCC,[2] high-risk serotypes of the human papilloma virus (HPV) have changed the epidemiology, especially of oropharynx cancer, in recent years.[3,4] Head and neck cancers arise via an accumulation of environmentally induced and inherent mutations in key signaling pathways, leading to immortalization, growth in the absence of growth signals, resistance to anti-growth signals, and the ability to avoid apoptosis, angiogenesis, invasion, and metastasis.[5,6] Our understanding of the genetic landscape of HNSCC has evolved over time and recently rapidly expanded with the results of recent genomic studies. As such, we are beginning to understand genetic drivers and accordingly develop precision medicine paradigms to treat this important and deadly disease. Here, we review key findings from genomic sequencing studies, identify gaps in data and need for further studies, and discuss the translational care potential from these studies.

## Early genetic studies

Prior to the genomics era, early studies attempted to identify key pathways in which alteration was thought to contribute to carcinogenesis in HNSCC, namely cell cycle dysregulation and constitutive cell proliferation. Early on, investigators discovered that loss of cell cycle regulation was an important driver for HNSCCs. Key genes involved in these pathways, namely *TP53*, *RB*, *CCND1*, *CDKN2A*, and *CDK4/6*, were determined to be mutated or otherwise aberrantly expressed in HNSCC.[6–12] Indeed, mutations in *TP53* are found in the majority of HPV-negative HNSCC

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cases.[8,9] Similarly, *CDKN2A* was noted to be inactivated by mutation or methylation in a large portion of HPV-negative HNSCCs,[11] and *CCND1* was found to be amplified in 80% of HNSCCs.[12] For HPV-positive HNSCC, the two virally encoded oncogenes, *E6* and *E7*, inactivate p53 and pRB, respectively, thus disrupting cell cycle regulation, and providing a mechanism for tumorigenesis.[13] A common thread between HPV-positive and HPV-negative tumors was the disruption of *TP53* function, either through mutation (HPV-negative tumors) or viral protein inactivation (HPV-positive tumors).

Similarly, early studies highlighted the importance of increased activation of cell growth and proliferation pathways in HNSCC. One of the earliest critical regulators of proliferation identified in HNSCC was *EGFR*, a transmembrane growth factor receptor that signals through the Ras–MAPK, PI3K–PTEN–AKT and phospholipase C pathways to promote cell proliferation (Fig. 1). Furthermore, EGFR can translocate to the nucleus and act as a transcription factor or co-activator of other transcription factors, such as STAT, leading to additional mechanisms for cell growth.[6,14] Importantly, early studies identified EGFR to be overexpressed or activated in a majority of cases of HNSCC, suggesting a critical role for this gene that would lead to future targeted therapeutic discoveries.[6,15] Other early studies identified frequent mutations and expression changes in other cell growth pathway genes, including *PIK3CA*, *PTEN*, and other growth factor receptors or their ligands.[16–21] Despite these important findings, however, studies were limited to single-gene analyses until the incorporation of newer sequencing techniques rapidly expanded our knowledge of the mutational landscape of HNSCCs.

## Insights from initial next generation sequencing studies

Given the complicated DNA landscape, multiple possible genetic alterations, and advancements in gene sequencing, researchers subsequently turned to next generation sequencing (NGS) tools to identify fundamental tumorigenic mechanisms for HNSCC (Table 1). Two key studies published in 2011 provided the initial mutational landscape of HNSCC by whole exome sequencing. Stransky et al. analyzed 74 HNSCC tumors (with normal tissue comparison),[22] and Agrawal et al. performed whole exome sequencing and gene copy number analyses on 32 HNSCCs.[23] Combined, these studies identified high rates of mutations in *TP53*, *PIK3CA*, and *CDKN2A*, consistent with early investigations into the genetic drivers of HNSCC. Interestingly, a high rate of inactivating *NOTCH1* mutations was verified, suggesting a tumor suppressor role for this gene. This had biologic plausibility given the role of *NOTCH1* in squamous differentiation (Fig. 1) and highlighted the utility of NGS tools in identifying previously unidentified genes associated with HNSCC. While limited in number, analysis of HPV positive patients suggested an lower overall mutational burden and different mutational spectrum in comparison to HPV negative tumors.

In a landmark study, The Cancer Genome Atlas (TCGA) profiled 279 HNSCCs with whole exome sequencing, copy

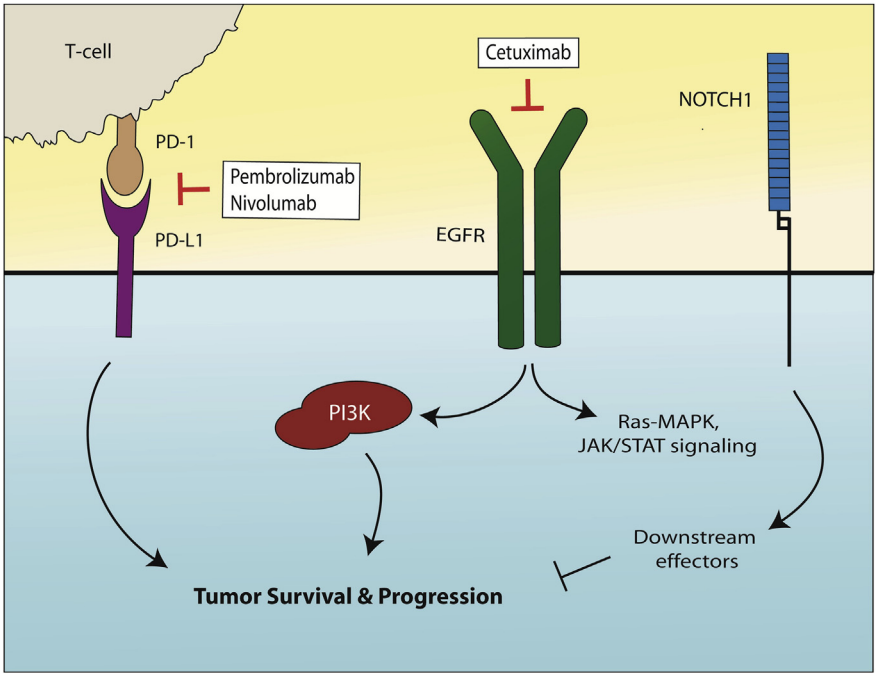


Figure 1. **Targetable pathways in HNSCC**. Currently FDA-approved targeted agents are limited to cetuximab (EGFR antibody), pembrolizumab and nivolumab (PD-1 antibodies). Additional targetable and commonly mutated pathways include other members of growth factor receptor families, Ras-MAPK and JAK/STAT downstream signaling pathways, PI3K, and downstream effectors of Notch1.