Squamous Cell Carcinoma

Subjects: Oncology

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More than 90% of all head and neck cancers (HNCs) are head and neck squamous cell carcinomas (HNSCCs) arising from the mucosal surfaces of the upper aerodigestive tract. HNSCCs are the the sixth most prevalent cancer worldwide, and are often associated with either carcinogens, such as alcohol and tobacco use, or oncogenic human papillomavirus (HPV) infection. HNSCCs have been found to be diverse with a high rate of genetic heterogeneity, resulting in hyper-activation of oncogenes (e.g., *PIK3CA* and *HRAS*) and loss-of-function mutations in tumor suppressor genes (e.g., *TP53*, *CASP8*, and *NOTCH1*). HNSCC cohorts from The Cancer Genome Atlas (TCGA) RNA-seq data and clinical data show patients with *PIK3CA* alterations, including amplification and gain, also have a higher chance of harboring *TP53* mutations. In addition, these patients bearing both mutations have a significantly worse 10-year survival prognosis compared with their wildtype cohort counterparts.

Keywords: HNSCC translational research ; HNSCC preclinical models ; Head and neck squamous cell carcinoma ; PIK3CA/TP53 ; TCGA ; gene mutations ; deletions ; amplifications ; Squamous Cell Carcinoma ; SCC

1. Introduction

Head and neck cancers (HNC) are a heterogeneous group of tumors arising from the mucosal surfaces of the upper aerodigestive tract [1]. Collectively, HNC is the sixth most prevalent cancer worldwide [1]. Some 90% of all HNCs are head and neck squamous cell carcinomas (HNSCCs) and HNSCCs are often associated with either carcinogens, such as alcohol and tobacco use, or oncogenic human papillomavirus (HPV) infection [2][3], thereby categorized as HPV(−) or HPV(+) HNSCCs. HNSCCs have been found to be diverse with a high rate of genetic heterogeneity, resulting in hyper-activation of oncogenes (e.g., *PIK3CA* and *HRAS*) and loss-of-function mutations in tumor suppressor genes (e.g., *TP53*, *CASP8*, and *NOTCH1*) [4][5]. Phosphoinositide 3-kinase (PI3K) is a frequently deregulated pathway in HNSCCs with a phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) gene mutation rate of approximately 16% and gene amplification of more than 30% in tumors [6][7]. PI3Ks are activated by receptor tyrosine kinases (RTKs), such as epidermal growth factor receptor (EGFR), and consist of different classes of enzymes vital for differentiation, proliferation and cell survival [8]. Mammalian target of rapamycin (mTOR) complexes (mTORC1 and mTORC2) and protein kinase B (also known as AKT) are also involved in this pathway that can activate transcription and other signaling molecules of the PI3K pathway [9]. Monoclonal antibodies (mAbs) that inhibit EGFR have been used for both HPV(−) and HPV(+) subtypes of HNSCCs; however, they were found to have limited efficacy and elicited resistance [10].

Another highly mutated gene in HNSSCs is the tumor protein p53 (*TP53*) gene, with over 80% of HPV(−) HNSCCs harboring loss-of-function mutations in *TP53*; however, *TP53* mutations occur much less frequently in HPV(+) HNSCCs (~3%) [4]. *TP53* is a tumor-suppressor gene encoding a transcription factor that maintains DNA repair, cell cycle, senescence and apoptosis [11]. These attributes make p53 an important cell sensor for oncogene activation and DNA damage. It has been found that the degradation of p53 is associated with HPV E6 oncoproteins [3]. Although there have been several therapies that target p53 in hopes to restore p53 function, they have yet to be proven effective in clinical trials [12]. By and large, *TP53* mutations are associated with poor HNSCC prognosis and overall survival with increased rate of recurrence and resistance to therapies. It remains poorly understood whether HNSCCs harboring different genetic alterations exhibit differential immune tumor microenvironment (TME). For instance, it is unknown whether HNSCCs with the double mutations in *TP53* and *PIK3CA* have a more immunosuppressive TME.

Prior studies have generated murine models that mimicked the alterations of *PIK3CA* or *p53* in HNSCCs. Transgenic mice that overexpressed wild-type PIK3CA in head and neck epithelium were generated; however, PIK3CA overexpression alone was not sufficient to initiate HNSCC formation [6]. Nevertheless, these PIK3CA Tg mice were much more susceptible to 4-nitroquinoline 1 oxide (4NQO)-induced HNSCC carcinogenesis [6]. Conditional deletion of p53 in mouse epithelial cells with K14.CrePR1 led to SCC development in about half of mice after 20 months [13]. To establish a mouse model that more closely resembles the genetic alterations in HNSCCs and allows us to better investigate immune evasion

mechanisms of HNSCCs, we generated a novel genetic model by deleting *p53* and constitutively activating *PIK3CA* in mouse keratin 15-expressing ($K15^{+}$) stem cells, which leads to the development of multilineage tumors including SCCs, termed Keratin-15-p53-PIK3CA (KPPA) tumors.

2. HNSCC Patients with Double Genetic Alterations in PIK3CA and TP53 Exhibited Worse Prognosis and More Immunosuppressive TME

The Cancer Genome Atlas (TCGA) RNA-seq data and clinical data of HNSCC cohorts were obtained from the cBioPortal (http://cbioportal.org). In analyzing the dataset of HNSCC patients (TCGA, PanCancer Atlas, *n* = 489 samples), we found that the patients with *PIK3CA* alterations, including amplification and gain, also have a higher chance of harboring *TP53* mutations (Figure 1A). After merging two datasets (see details in Methods), we divided the patients into four different groups: $PIK3CA^{Amp}/TP53^{Mutated}$ (*n* = 294), $PIK3CA^{Amp}/TP53^{WT}$ (*n* = 85), $PIK3CA^{WT}/TP53^{Mutated}$ (*n* = 56), and $PIK3CA^{WT}/TP53^{WT}$ (*n* = 54). Kaplan Meier curves of five-year survival were shown for four different groups, and $PIK3CA^{Amp}/TP53^{Mutated}$ group exhibited a hazard ratio of 1.61 (95% CI, 0.94–2.75) compared to $PIK3CA^{WT}/TP53^{WT}$ group (Figure 1B). $PIK3CA^{Amp}/TP53^{Mutated}$ group's 10-year survival hazard ratio was 1.8 (95% CI, 1.06–3.07), and showed a significantly worse prognosis than $PIK3CA^{WT}/TP53^{WT}$ group.

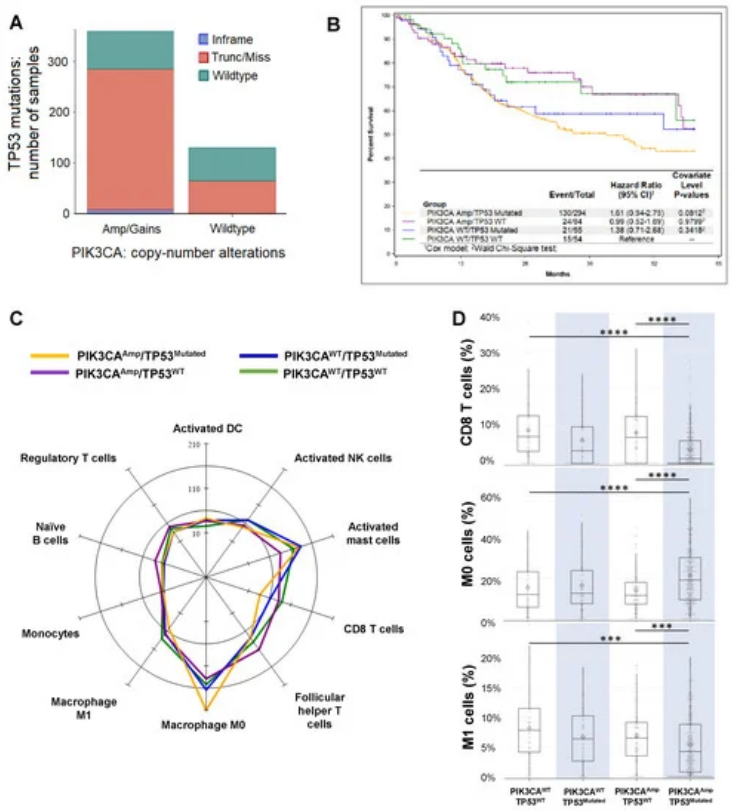

Figure 1. Analysis of The Cancer Genome Atlas (TCGA) datasets of Head and Neck Squamous cell carcinoma (HNSCC) patients. (**A**) Association between PIK3CA gene alterations and TP53 gene mutation in HNSCC patients (TCGA, PanCancer Atlas, *n* = 489 samples). The Cancer Genome Atlas (TCGA) RNA-seq data and clinical data of HNSCC cohorts were obtained from the cBioPortal (http://cbioportal.org). Patients with $PIK3CA^{Amp/gain}$ (*n* = 359) had a higher chance to harbor TP53 mutations than patients with $PIK3CA^{WT}$ (*n* = 130). (**B**) Kaplan-Meier overall 5-year survival curves of HNSCC patients in 4 groups ($PIK3CA^{Amp}/TP53^{Mutated}$, $PIK3CA^{Amp}/TP53^{WT}$, $PIK3CA^{WT}/TP53^{Mutated}$, and $PIK3CA^{WT}/TP53^{WT}$) as described in Methods. Only patients with available survival data were included for this analysis (*n* = 487). (**C**) A radar plot of the cell types that reached significance in the omnibus Kruskal-Wallis test when comparing among the 4 groups. The scale is per 1000 cells. (**D**) Box and whisker plots of CD8 T cells, resting macrophages (M0), and M1 macrophages. The expression of CD8 T cell signature genes: $PIK3CA^{Amp}/TP53^{Mutated}$ group (3.77 ± 5.32) was significantly lower than groups of $PIK3CA^{WT}/TP53^{WT}$ (8.97 ± 7.86) and $PIK3CA^{Amp}/TP53^{WT}$ (8.22 ± 8.12). The expression of M0 signature genes: $PIK3CA^{Amp}/TP53^{Mutated}$ group (21.07 ± 14.08) was significantly higher than groups of $PIK3CA^{WT}/TP53^{WT}$ (15.25 ± 12.32) and $PIK3CA^{Amp}/TP53^{WT}$ (13.93 ± 11.23). The expression of M1 signature genes: $PIK3CA^{Amp}/TP53^{Mutated}$ group (5.35 ± 5.05) was significantly lower than groups of $PIK3CA^{WT}/TP53^{WT}$ (8.17 ± 5.62) and $PIK3CA^{Amp}/TP53^{WT}$ (6.92 ± 5.17).

We uploaded RNA-seq data of HNSCC patients onto CIBERSORT (see details in Methods), which estimated the relative proportions of 22 immune cell types, with a more in-depth dissection shown in Supplemental Table S1. Both innate and adaptive immune cells varied in their expression levels depending on the genetic alterations in 4 groups (Figure 1C). In particular, we found that the expression of CD8 T cell signature genes was significantly lower in PIK3CA^{Amp}/TP53^{Mutated} group, compared with PIK3CA^{WT}/TP53^{WT} and PIK3CA^{Amp}/TP53^{WT} groups (Figure 1D). In addition, PIK3CA^{Amp}/TP53^{Mutated} group had significantly lower expression of activated NK cell-associated genes compared with PIK3CA^{WT}/TP53^{WT} and PIK3CA^{WT}/TP53^{Mutated} groups. PIK3CA^{Amp}/TP53^{Mutated} group expressed significantly higher level of resting macrophage (M0) signature genes but lower level of activated macrophage (M1) genes than PIK3CA$^{WT/}$TP53^{WT} and PIK3CA^{Amp}/TP53^{WT} groups (Figure 1D). We conclude that HNSCCs with the genotype of PIK3CA^{Amp}/TP53^{Mutated} appear to have a highly immunosuppressive TME.

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