Correlate the TP53 Mutation and the HRAS Mutation with Immune Signatures in Head and Neck Squamous Cell Cancer

Haoyu Lu a,b,c,1, Mengyuan Li a,b,c,1, Zehang Jiang a,b,c, Zhixian Liu a,b,c, Xiaosheng Wang a,b,c,*

* Biomedical Informatics Research Lab, School of Basic Medicine and Clinical Pharmacy, China Pharmaceutical University, Nanjing 211198, China
† Cancer Genomics Research Center, School of Basic Medicine and Clinical Pharmacy, China Pharmaceutical University, Nanjing 211198, China
‡ Big Data Research Institute, China Pharmaceutical University, Nanjing 211198, China

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A B S T R A C T

Although immunotherapy has emerged as an effective therapeutic strategy for various cancers including head and neck squamous cell carcinomas (HNSCCs), only a subset of patients can benefit from such therapy. Hence, it is pressing to discover predictive biomarkers for cancer immunotherapy response. TP53 and HRAS mutations frequently occur in HNSCC and correlate with a worse prognosis in HNSCC. We extensively characterized the associations of TP53 mutations and HRAS mutations with HNSCC immunity based on multiple cancer genomics datasets. We compared the enrichment levels of 20 immune signatures between TP53-mutated and TP53-wildtype HNSCCs, and between HRAS-mutated and HRAS-wildtype HNSCCs, and found that TP53 mutations were associated with depressed immune signatures while HRAS mutations were associated with enhanced immune signatures in HNSCC. Moreover, we found multiple p53- and RAS-mediated pathways showing significant correlations with HNSCC immunity. Furthermore, we demonstrated that the association between TP53 mutation and tumor immunity was independent of the human papillomavirus (HPV) infection and smoking status in HNSCC. These data suggest that p53 and RAS may play important roles in regulating HNSCC immunity and that the TP53 and HRAS mutation status could be useful biomarkers for stratifying HNSCC patients responsive to immunotherapy.

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1. Introduction

Head and neck cancer is the fifth most common cancer worldwide, most of which are squamous cell carcinomas (HNSCCs) [1]. HNSCC has a poor prognosis once the disease is not amenable to surgery, relapses or becomes metastatic [2]. Recently, cancer immunotherapy has achieved rapid clinical successes in treating multiple cancers including HNSCC [3]. Thus, the immunotherapy could be a promising treatment option for the HNSCC patients who have failed to surgery, radiation or chemotherapy. Unfortunately, thus far only approximately 20% cancer patients can benefit from immunotherapy such as immune checkpoint blockades [4]. A series of studies have focused on identifying molecular features that are associated with cancer immunotherapy response, such as tumor mutation burden (TMB), deficient mismatch-repair (dMMR), neoantigens, and PD-L1 expression in tumor cells [5–10]. A few studies have explored the associations of gene mutations with cancer immunotherapy response, e.g., the associations of TP53 and KRAS mutations with immunotherapy response in lung cancer [11].

TP53 mutations frequently occur in cancer and are associated with poor prognosis in a wide variety of cancers [12]. In particular, TP53 is the most frequently mutated gene in HNSCC and TP53-mutated HNSCCs have a worse overall survival (OS) prognosis than TP53-wildtype HNSCCs [13]. Myriad studies have shown that p53 plays an important role in tumor suppression via promoting cell cycle arrest and apoptosis [14]. A few studies have associated p53 with tumor immune regulation [15–18]. For example, p53 played a role in antitumor immunosurveillance by regulating VISTA [15], p53 activation could enhance antitumor immunity [18]. The oncogenes of RAS family (KRAS, HRAS, and NRAS) are frequently mutated in various cancers and are associated unfavorable clinical outcomes in cancer [19]. Several studies have shown that the RAS signaling could promote tumor immunosuppression [20–22].
Since deregulation of the p53 and RAS pathways are significantly associated with tumor development and immune evasion, a comprehensive investigation of their associations with tumor immunity in HNSCC is worthwhile considering that such exploration remains lacking. To this end, we explored the associations of TP53 mutations and RAS mutations with HNSCC immunity. We compared the enrichment levels of 20 immune signatures between TP53-mutated and TP53-wildtype HNSCCs, and between HRAS-mutated and HRAS-wildtype HNSCCs (we did not analyze KRAS and NRAS since both genes are rarely mutated in HNSCC) using several HNSCC multi-omics datasets [13,23]. Furthermore, we explored the phenotype and molecular features that were associated with the differential immune signatures between TP53 (or HRAS) mutated and wildtype HNSCCs. This study aimed to identify biomarkers potentially effective for predicting responses to HNSCC immunotherapy.

2. Results

2.1. TP53 and HRAS Mutations are Associated with Depressed and Elevated Immune Signatures in HNSCC, Respectively

We found that 18 out of the 20 immune signatures analyzed showed significantly lower enrichment levels in TP53-mutated HNSCCs than in TP53-wildtype HNSCCs (Mann-Whitney U test, \( P < 0.05 \)) (Fig. 1A; Supplementary Table S1). Moreover, TP53-mutated HNSCCs had significantly lower immune scores than TP53-wildtype HNSCCs (Mann-Whitney U test, \( P = 4.25 \times 10^{-7} \)) (Fig. 1B). In contrast, 18 of the 20 immune signatures showed significantly higher enrichment levels in HRAS-mutated HNSCCs than in HRAS-wildtype HNSCCs (Mann-Whitney U test, \( P < 0.05 \)) (Supplementary Table S2), and HRAS-mutated HNSCCs had significantly higher immune scores (Mann-Whitney U test, \( P = 0.005 \)) (Fig. 1C). Altogether, these data indicated that TP53 mutations correlated with depressed tumor immunity, while HRAS mutations correlated with elevated tumor immunity in HNSCC.

Moreover, numerous marker genes of immune signatures showed decreased expression levels in TP53-mutated HNSCCs versus TP53-wildtype HNSCCs (Supplementary Tables S3–S7). For example, 80% of the B cell markers, the CD8+ T cell marker (CD8A), all NK cell markers, both cytolytic activity markers (GZMA and PRF1), and 104 (87%) of the 120 tumor-infiltrating lymphocytes (TILs) markers showed reduced expression in TP53-mutated HNSCCs relative to TP53-wildtype HNSCCs. In contrast, a considerable number of immune signature marker genes showed markedly increased expression in HRAS-mutated HNSCCs compared to HRAS-wildtype HNSCCs (Supplementary Tables S3–S7). For example, the CD8+ T cell marker and both cytolytic activity markers were more highly expressed in HRAS-mutated HNSCCs than in HRAS-wildtype HNSCCs. Of the 15 pro-inflammatory genes (CD88, TBX21, CD19, IFNG, IL12B, PRF1, IL12A, CXCL10, CXCL9, CXCL13, CCL5, GZMB, and IFNγ) [24], 14 (except STAT1) showed significantly lower expression levels in TP53-mutated HNSCCs than in TP53-wildtype HNSCCs (Student’s t-Test, false discovery rate: FDR < 0.05) (Supplementary Table S7). In contrast, 10 of the 15 pro-inflammatory genes showed significantly higher expression levels in HRAS-mutated HNSCCs than in HRAS-wildtype HNSCCs (Supplementary Table S7). Notably, GZMB (granzyme B) and PRF1 (perforin 1) whose products are mainly secreted by NK cells and cytotoxic T lymphocytes [25], were downregulated in TP53-mutated HNSCCs versus TP53-wildtype HNSCCs, while were upregulated in HRAS-mutated HNSCCs versus HRAS-wildtype HNSCCs.

Fig. 1. TP53-mutated HNSCCs have reduced immune activity compared to TP53-wildtype HNSCCs while HRAS-mutated HNSCCs have increased immune activity compared to HRAS-wildtype HNSCCs. A. Heatmap showing the enrichment levels (ssGSEA scores) of 20 immune signatures in TP53-mutated HNSCCs versus TP53-wildtype HNSCCs. ssGSEA: single-sample gene-set enrichment analysis [49,50]. B. The immune infiltration levels (immune scores evaluated by ESTIMATE [53]) are significantly lower in TP53-mutated HNSCCs than in TP53-wildtype HNSCCs (Mann-Whitney U test, \( P < 0.01 \)). C. The immune infiltration levels are significantly higher in HRAS-mutated HNSCCs than in HRAS-wildtype HNSCCs. D. GSEA [26] identifies numerous immune-related KEGG [52] pathways downregulated in TP53-mutated HNSCCs versus TP53-wildtype HNSCCs and immune-related pathways upregulated in HRAS-mutated HNSCCs versus HRAS-wildtype HNSCCs. E. The rations between immune-stimulatory signatures and immune-inhibitory signatures are significantly lower in TP53-mutated HNSCCs than in TP53-wildtype HNSCCs while significantly higher in HRAS-mutated HNSCCs than in HRAS-wildtype HNSCCs (Mann-Whitney U test P-values are shown), M1: M1 macrophages, M2: M2 macrophages.
and ease, hematopoietic cell lineage, asthma, T cell receptor signaling, natural killer cell mediated cytotoxicity, allograft rejection, toll-like receptor signaling (Fig. 1D). In contrast, numerous immune-related pathways were significantly upregulated in HNSCCs compared to HNSC-wildtype HNSCCs, including cytokine-cytokine receptor interaction, antigen processing and presentation, natural killer cell mediated cytotoxicity, toll-like receptor signaling, and the positive correlation between TP53 mutation and immune cytolytic activity and immune signatures (Supplementary Table S6). In contrast, 13 (54%) and zero HLA mutations were significantly downregulated in TP53-wildtype HNSCCs versus in TP53-wildtype HNSCCs (Mann-Whitney U test, \(P = 0.001\)) while showed no significant difference between HRA5-mutated and HRA5-wildtype HNSCCs (Mann-Whitney U test, \(P = 0.621\)). These results suggest that the reduced immunogenic activity in TP53-mutated HNSCCs may be ascribed to the depressed HLA function, but not to TMB or mutation-associated neoantigens. Interestingly, the aneuploidy levels were significantly higher in TP53-mutated HNSCCs than in TP53-wildtype HNSCCs (Mann-Whitney U test, \(P = 5.50 \times 10^{-5}\)) while had no significant differences between HRA5-mutated and HRA5-wildtype HNSCCs (Mann-Whitney U test, \(P = 0.873\)) (Fig. 3B). These findings are in line with previous studies showing that tumor aneuploidy correlated with reduced tumor immunity in cancer [29].

2.4. Prediction of Immune Signatures by TP53 Mutation, HRAS Mutation, TMB, and Tumor Aneuploidy in HNSCC

Based on the logistic regression model with four predictors (TP53 mutation, HRAS mutation, TMB, and aneuploidy), we evaluated the contribution of TP53 mutation and HRAS mutation in predicting immune cytolytic activity and immune infiltration levels (immune score) in HNSCC. We found that three of the four predictors significantly predicted immune cytolytic activity, including TP53 mutation (\(\beta\) coefficient: \(\beta = -1.214, P = 1.96 \times 10^{-4}\)), HRAS mutation (\(\beta = 1.619, P = 0.018\)), and aneuploidy (\(\beta = -0.664, P = 0.001\)) (Fig. 4). As expected, both TP53 mutation and aneuploidy were negative predictors and HRAS mutation was a positive predictor for immune cytolytic activity. In predicting immune infiltration levels, both TP53 mutation (\(\beta = -1.085, P = 6.95 \times 10^{-4}\)) and aneuploidy (\(\beta = -0.620, P = 0.002\)) were significant negative predictors, and HRAS mutation was a positive predictor (\(\beta = 0.650, P = 0.257\)) (Fig. 4). These results confirmed the negative correlation between TP53 mutations and immune signatures and the positive correlation between HRAS mutations and immune signatures in HNSCC. Meanwhile, these results confirmed the significant negative correlation between aneuploidy and immune signatures in cancer [29]. Interestingly, TMB showed minor contribution in predicting immune cytolytic activity (\(\beta = 0.001, P = 0.134\)) and immune infiltration levels (\(\beta = 1.10 \times 10^{-4}, P = 0.794\)) in HNSCC. It indicates that TMB is not significant in determining tumor immunity in HNSCC.

2.5. Identification of the Pathways that are Altered by TP53 or HRAS Mutations and are Significantly Associated with Immune Signatures in HNSCC

GSEA [26] showed that not only the immune-related pathways but also a number of cancer-associated pathways were disturbed upon TP53 or HRAS mutations in HNSCC. These pathways included the p53, cell cycle, apoptosis, mismatch repair, Jak-STAT, focal adhesion, ECM-receptor interaction, calcium, MAPK, PI3K-Akt, mTOR, RAS, Wnt, Hedgehog, TGF-beta, ErbB, and glycolysis pathways. Interestingly, we found that most of these pathways were significantly associated with immune signatures in HNSCC (Fig. 5A, B). Notably, as a tumor suppressor, p53 prominently functions on promoting cell cycle arrest and apoptosis [14]. Accordingly, TP53 mutations could lead to downregulation of the p53 and apoptosis pathways and upregulation of the cell cycle pathway in cancer. Our results showed that almost all 20 immune signatures

HLA genes encode MHC proteins which are involved in the regulation of the immune system in humans [27]. Of 24 HLA genes analyzed, 15 (62.5%) and zero were downregulated and upregulated in TP53-mutated HNSCCs compared to TP53-wildtype HNSCCs, respectively (Fig. 2A; Supplementary Table S6). In contrast, 13 (54%) and zero HLA genes were more highly and lowly expressed in HRA5-mutated HNSCCs than in HRA5-wildtype HNSCCs, respectively (Fig. 2B; Supplementary Table S6). These results suggest that TP53 mutations may inhibit HLA expression while HRAS mutations may promote HLA expression in HNSCC. The finding of TP53 mutations repressing HLA expression in HNSCC is in line with a previous report that p53 could increase expression of MHC proteins in cancer [28].

2.3. Correlations of TP53 Mutations and HRAS Mutations with TMB and Tumor Aneuploidy

TMB and tumor aneuploidy have been shown to significantly correlate with tumor immunity [29]. We found that TP53-mutated HNSCCs had significantly higher TMB than TP53-wildtype HNSCCs (Mann-Whitney U test, \(P = 2.85 \times 10^{-7}\)) while HRAS mutations had no significant correlation with TMB in HNSCC (Mann-Whitney U test, \(P = 0.522\)) (Fig. 3A). Moreover, gene mutations may yield neoantigens that are associated with tumor immunity [30]. We found that the mutations yielding predicted HLA-binding peptides [25] were much more in TP53-mutated HNSCCs than in TP53-wildtype HNSCCs (Mann-Whitney U test, \(P = 0.014\)) while showed no significant difference between HRAS-mutated and HRAS-wildtype HNSCCs (Mann-Whitney U test, \(P = 0.621\)). These results suggest that the reduced immunogenic activity in TP53-mutated HNSCCs may be ascribed to the depressed HLA function, but not to TMB or mutation-associated neoantigens. Interestingly, the aneuploidy levels were significantly higher in TP53-mutated HNSCCs than in TP53-wildtype HNSCCs (Mann-Whitney U test, \(P = 5.50 \times 10^{-5}\)) while had no significant differences between HRAS-mutated and HRAS-wildtype HNSCCs (Mann-Whitney U test, \(P = 0.873\)) (Fig. 3B). These findings are in line with previous studies showing that tumor aneuploidy correlated with reduced tumor immunity in cancer [29].

2.2. TP53 and HRAS Mutations are Associated with Reduced and Increased Expression of Human Leukocyte Antigen (HLA) Genes in HNSCC, Respectively

Overall, these findings suggest that TP53 mutations may inhibit inflammatory and immune activity while HRAS mutations may promote them in HNSCC.

Furthermore, GSEA [26] analysis revealed that numerous immune-related pathways were significantly downregulated in TP53-mutated HNSCCs compared to TP53-wildtype HNSCCs. These pathways included antigen processing and presentation, allograft rejection, asthma, autoimmune thyroid disease, B cell receptor signaling, chemokine signaling, cytokine-cytokine receptor interaction, hematopoietic cell lineage, intestinal immune network for IgA production, leukocyte transendothelial migration, natural killer cell mediated cytotoxicity, primary immunodeficiency, systemic lupus erythematosus, T cell receptor signaling, and toll-like receptor signaling (Fig. 1D). In contrast, numerous immune-related pathways were significantly upregulated in HRA5-mutated HNSCCs compared to HRA5-wildtype HNSCCs, including cytokine-cytokine receptor interaction, antigen processing and presentation, natural killer cell mediated cytotoxicity, allograft rejection, toll-like receptor signaling, chemokine signaling, autoimmune thyroid disease, hematopoietic cell lineage, asthma, T cell receptor signaling, chronic myeloid leukemia, and intestinal immune network for IgA production (Fig. 1D). These results again demonstrate that TP53 mutations are associated with depressed immune signatures while HRAS mutations are associated with enhanced immune signatures in HNSCC.

Interestingly, we observed that the ratio between immune-stimulatory cells (CD8+ T cells with marker gene CDA8) and immune-inhibitory cells (CD4+ regulatory T cells with marker genes CTLA4, FOXP3, GPR15, IL32, IL4, and IL5) was significant reduced in TP53-mutated HNSCCs versus TP53-wildtype HNSCCs (Mann-Whitney U test, \(P = 1.95 \times 10^{-13}\)) (Fig. 1E). A significant decrease in the ratio between pro-inflammatory cytokines (marker genes IFNG, IL-1A, IL-1B, and IL-2) and anti-inflammatory cytokines (IL-4, IL-10, IL-11, and TGF81) was also observed in TP53-mutated HNSCCs versus TP53-wildtype HNSCCs (Mann-Whitney U test, \(P = 4.84 \times 10^{-9}\)) (Fig. 1E). The ratio between immune-inciting M1 macrophages (CD64, IDO, SOCS1, and CXCL10) and immune-inhibiting M2 macrophages (MRC1, TGM2, CD23, and CCL22) was significantly reduced in TP53-mutated HNSCCs versus TP53-wildtype HNSCCs (Mann-Whitney U test, \(P = 3.15 \times 10^{-7}\)) (Fig. 1E). However, these ratios were significantly increased in HRA5-mutated HNSCCs versus HRA5-wildtype HNSCCs (Fig. 1E). These results again suggest that TP53 mutations and HRAS mutations are associated with a reduced and an increased immune/inflammation activity in HNSCC.

In another HNSCC multi-omics dataset GSE65858 [23], we observed that multiple immune signatures were significantly downregulated in TP53-mutated HNSCCs versus TP53-wildtype HNSCCs, e.g., CD8+ T cells, immune cytolytic activity, and immune score (Supplementary Fig. S1). It verified that TP53 mutations were associated the reduced immune activity in HNSCC.
Fig. 2. Comparisons of the expression levels of human leukocyte antigen (HLA) genes between TP53-mutated and TP53-wildtype HNSCCs, and between HRAS-mutated and HRAS-wildtype HNSCCs. A. A number of HLA genes have significantly lower expression levels in TP53-mutated HNSCCs than in TP53-wildtype HNSCCs (Student’s t-Test P-values are shown). B. A number of HLA genes have significantly higher expression levels in HRAS-mutated HNSCCs than in HRAS-wildtype HNSCCs.

Fig. 3. Correlations of TP53 mutations and HRAS mutations with tumor mutation burden (TMB) and tumor aneuploidy. A. TP53-mutated HNSCCs have significantly higher TMB than TP53-wildtype HNSCCs while HRAS mutations have no significant correlation with TMB in HNSCC (Mann-Whitney U test P-values are shown). B. TP53-mutated HNSCCs have significantly higher aneuploidy levels than TP53-wildtype HNSCCs while HRAS mutations have no significant correlation with tumor aneuploidy in HNSCC (Mann-Whitney U test P-values are shown). TMB is the total somatic mutation count in tumor and tumor aneuploidy is the tumor ploidy score evaluated by ABSOLUTE [54].
Fig. 4. Logistic regression analysis shows that TP53 mutation and aneuploidy were negative predictors and HRAS mutation was a positive predictor for immune signatures in HNSCCs. β value: β coefficient.

Fig. 5. Immune signatures are significantly associated with p53-mediated or RAS-mediated pathways in HNSCC. A. Immune signatures are significantly associated with p53-mediated pathways in HNSCC. B. Immune signatures are significantly associated with RAS-mediated pathways in HNSCC. The size of circles is proportional to the absolute values of correlation coefficients. C. Logistic regression analysis shows that the cell cycle score was a negative predictor and the apoptosis score was a positive predictor in predicting immune signatures in HNSCCs. The cell cycle score and apoptosis score were the ssGSEA scores [49,50] of the gene sets in the cell cycle and apoptosis pathways.
significantly positively correlated with the p53 and apoptosis pathways and inversely correlated with the cell cycle pathway (Fig. 5A). It conforms to our finding that TP53 mutations were associated with depressed immune signatures in HNSCC. On the other hand, as an oncogene of RAS family, HRAS mutations may lead to hyperactivation of the RAS pathways. We found that almost all 20 immune signatures showed a significant positive correlation with the RAS pathways (Fig. 5B), consistent with our finding that HRAS mutations were associated with elevated immune signatures in HNSCC. Intriguingly, pro-oncogenic pathways were likely to exhibit a negative correlation with immune signatures in HNSCC, e.g., the cell cycle, mTOR, RAS, Wnt, Hedgehog, TGF-beta, ErbB, and glycolysis pathways (Fig. 5A, B). It indicates that the hyperactivation of pro-oncogenic pathways may promote tumor immunosuppression. Altogether, these results suggest that TP53 mutations and HRAS mutations may alter the activity of their mediated pathways, thereby contributing to the depressed and elevated immune signatures in TP53-mutated HNSCCs and HRAS-mutated HNSCCs, respectively.

To further prove that the negative association between TP53 mutations and tumor immunity in HNSCC is associated with the cell cycle and apoptosis pathways, we used a logistic regression model with the predictors of cell cycle score and apoptosis score to predict immune signatures (immune cytolytic activity and immune infiltration levels). We found that the cell cycle score was a negative predictor and the apoptosis score was a positive predictor in predicting these immune signatures (Fig. 5C). These results indicate that TP53 mutations lead to cell cycle activation and apoptosis inhibition, which in turn affect tumor immunity.

2.6. Identification of Proteins Whose Expression is Associated with TP53 Mutations as well as Immune Infiltration in HNSCC

We identified 8 proteins (Syk, Caspase-7, Cyclin B1, TIGAR, Bcl-2, Lck, VEGFR2, and PCNA) and 2 proteins (CK5 and Rb) having significantly lower and higher expression levels in TP53-mutated HNSCCs than in TP53-wildtype HNSCCs, respectively (Student’s t-Test, FDR < 0.2). Of these proteins, Syk, Caspase-7, Bcl-2, and Lck had a significant positive expression correlation with immune infiltration levels (immune scores) in HNSCC (Spearman correlation, P < 0.001) (Fig. 6). Syk (spleen tyrosine kinase) is a non-receptor cytoplasmic enzyme that is primarily expressed in cells of hematopoietic lineage and regulates the biological processes that are associated with innate and adaptive immunity [31]. This protein functions as a tumor suppressor and is a p53 target [32,33]. The downregulation of Syk may be associated with the p53 dysfunction and contribute to the immunosuppression in the TP53-mutated HNSCC subtype. Lck is a member of the Src tyrosine kinase family and plays a key role in regulation of developing T-cells [34]. The downregulation of Syk may contribute to the depressed antitumor immunity
in TP53-mutated HNSCCs. Caspase-7 and Bcl-2 are importantly involved in p53-regulated apoptosis [35,36]. The downregulation of both proteins indicated the reduced apoptosis activity that was associated the depressed immune activity in TP53-mutated HNSCCs. Taken together, these results demonstrate that the differential immune activity is significantly associated with the differential immune-associated protein expression between TP53-mutated and TP53-wildtype HNSCCs.

2.7. Immune Signatures are Positively Associated with Survival Prognosis in HNSCC

Survival analyses showed that the elevated enrichment of 12 immune signatures consistently correlated with better OS and/or DFS in HNSCC (log-rank test, \( P < 0.05 \)) (Fig. 7A). These 12 immune signatures included B cells, CD4+ regulatory T cells, CD8+ T cells, neutrophils,
in HNSCC. have a strong inverse correlation regardless of the HPV infection status.

HNSCCs could be due to the lower HPV infection rate relative to anti-PD-1/PD-L1/CTLA-4 immunotherapy, we examined the correlation between TP53 mutations and OS prognosis. We found that TP53-mutated HNSCCs had worse OS than TP53-wildtype HNSCCs (log-rank test, \( P = 0.050 \)) (Fig. 7C). The negative correlation between TP53 mutations and OS in the immunotherapy setting could be attributed to the unfavorable response to immunotherapy in TP53-mutated HNSCCs compared to TP53-wildtype HNSCCs. In the same cohort, because all HNSCCs were HRAS-wildtype, we did not analyze the correlation between HRAS mutations and survival prognosis.

2.8. The Negative Association between TP53 Mutations and Tumor Immunity is Independent of the Human Papillomavirus (HPV) Infection and Smoking Status in HNSCC

HPV and smoking are important factors in the rise of non-smoker HNSCCs and smoker HNSCCs, respectively [13,41]. We found that TP53-mutated HNSCCs had a significantly lower rate of HPV infection than TP53-wildtype HNSCCs (7% versus 46%, Fisher’s exact test, \( P = 1.60 \times 10^{-22} \), odds ratio: OR = 0.090). As expected, HPV+ HNSCCs likely had increased immune activity compared to HPV- HNSCCs (Supplementary Table S8). Thus, the lower immune activity in TP53-mutated HNSCCs could be due to the lower HPV infection rate relative to TP53-wildtype HNSCCs. Nevertheless, we found that a majority of the immune signatures showed significantly lower enrichment levels in TP53-mutated HPV- HNSCCs versus TP53-wildtype HPV- HNSCCs, as well as in TP53-mutated HPV+ HNSCCs versus TP53-wildtype HPV+ HNSCCs (Supplementary Table S9). To further investigate how the association between TP53 mutations and tumor immunity is affected by the HPV infection factor, we constructed a logistic regression model with the predictors of TP53 mutation and HPV infection to predict immune signatures (immune cytolytic activity and immune infiltration levels). We found that TP53 mutation was a significant negative predictor in predicting both immune signatures (\( \beta = -1.070 \) and \( P = 0.003 \) in predicting immune cytolytic activity; \( \beta = -0.868 \) and \( P = 0.019 \) in predicting immune infiltration levels) (Fig. 8). All together, these analyses consistently demonstrate that TP53 mutations and tumor immunity have a strong inverse correlation regardless of the HPV infection status in HNSCC.

In addition, we found that TP53-mutated HNSCCs contained a higher proportion of heavy smokers compared to TP53-wildtype HNSCCs (Fisher’s exact test, \( P = 0.043 \), OR = 1.582). Unexpectedly, we found that heavy-smoker HNSCCs were inclined to have depressed immune activity compared to light-smoker and non-smoker HNSCCs (Supplementary Table S10), although the former had significantly higher TMB than the latter (Mann-Whitney U test, \( P = 5.11 \times 10^{-16} \)). This suggests that the heavy-smoking may dampen the immune function of HNSCCs.

Furthermore, we found that most of the immune signatures had significantly lower enrichment levels in TP53-mutated heavy-smoker HNSCCs versus TP53-wildtype heavy-smoker HNSCCs, as well as in TP53-mutated non-heavy-smoker HNSCCs versus TP53-wildtype non-heavy-smoker HNSCCs (Supplementary Table S11). It demonstrates that the smoking factor solely cannot explain the differential immune activity between TP53-mutated and TP53-wildtype HNSCCs.

3. Discussion

TP53 has a high mutation rate (>70%) in HNSCC and the TP53 mutation is associated with a worse prognosis in HNSCC [12]. Strikingly, we found that almost all the immune signatures analyzed showed significantly lower activities in TP53-mutated HNSCCs versus TP53-wildtype HNSCCs. In contrast, the mutation of HRAS, which is the most frequently mutated RAS gene in HNSCC (~6%), was linked with higher immune activities in HNSCC. These results demonstrate the opposite effect of the tumor suppressor gene (TP53) mutation and the oncogene (HRAS) mutation on the tumor immune microenvironment (TIM) in HNSCC. It has been shown that high immune signatures in the TIM, e.g., dense infiltration of lymphocytes such as CD8+ T cells and B cells, often indicate favorable clinical outcomes and active response to immunotherapy in cancer patients [37,38]. Thus, our data have potential clinical implications that TP53-wildtype HNSCCs more likely respond to immunotherapy than TP53-mutated HNSCCs and that HRAS-mutated HNSCCs may be more responsive to immunotherapy as compared to HRAS-wildtype HNSCCs.

TP53 mutations often result in the deregulation of p53 function in regulating cell cycle, apoptosis, and genome stability [14,42]. Thus, TP53-mutated HNSCCs likely have increased cell cycle, reduced apoptosis, and high genome instability. Our results and reports from other studies [29,43,44] consistently showed that cell cycle inhibited tumor immunity, apoptosis promoted tumor immunity, and genome instability suppressed tumor immunity. Thus, the depressed tumor immunity in TP53-mutated HNSCCs could be attributed to the deregulation of

Fig. 9. TP53-mutations result in the deregulation of p53-mediated cell cycle, apoptosis, and genome instability thereby contributing to the depressed immune activity in HNSCC.
these p53 functions (Fig. 9). Moreover, we found plentiful p53- and RAS-mediated pathways whose activity was significantly associated with immune activity in HNSCC (Fig. 5). These pathways are involved in various cancer-associated activities, including DNA damage repair, proliferation, metabolism, inflammation, epithelial-mesenchymal transition (EMT), angiogenesis, and metastasis, suggesting that the disturbance of a wide range of cancer-associated pathways may alter the TIM in cancer and that the effective intervention of these pathways may enhance antitumor immunity and cancer immunotherapy response.

We found that HRAS mutations were associated with elevated antitumor immune signatures in HNSCC. However, the HRAS mutation was not associated with a better survival prognosis in the TCGA HNSCC cohort (log-rank test, \( P = 0.516, 0.632 \) for OS and DFS, respectively). A possible explanation is that HRAS mutations also promoted immunosuppressive signatures, such as PD-L1 expression. However, we believe that the HRAS mutation could be a predictive biomarker for favorable response to anti-PD-1/PD-L1 immunotherapy since both the elevated antitumor immune infiltration \([45]\) and PD-L1 expression \([46]\) indicate a more active response to anti-PD-1/PD-L1 inhibitors.

The relationship between gene mutations and tumor immunity could vary with tumor progression. We compared the enrichment levels of the 20 immune signatures between TP53-mutated and TP53-wildtype HNSCCs within early- and late-stage cancers, respectively. We found that 18 immune signatures displayed significantly lower enrichment levels in TP53-mutated early-stage HNSCCs than in TP53-wildtype early-stage HNSCCs, and in TP53-mutated late-stage HNSCCs than in TP53-wildtype late-stage HNSCCs (Mann-Whitney U test, \( P < 0.05 \)). It suggests that the association between TP53 mutations and tumor immunity does not vary with tumor progression.
4.2. Comparisons of Gene Expression Levels, Immune Signature Enrichment Levels, and Protein Expression Levels Between Two Classes of Samples

For HNSCC gene expression profiling data, we normalized the gene expression values by base-2 log transformation and compared the expression levels of a single gene between two classes of samples using Student’s $t$-Test. We quantified the enrichment level of an immune signature represented by a gene set in a sample by the single-sample gene-set enrichment analysis (ssGSEA) score [49,50] and compared the enrichment levels (ssGSEA scores) of an immune signature between two classes of samples using Mann-Whitney $U$ test. We compared the ratios between immune-stimulatory signatures and immune-inhibitory signatures between two classes of samples based on the ratios between the average expression levels of marker genes of immune-stimulatory signatures and the average expression levels of marker genes of immune-inhibitory signatures (CD8+/CD4+ Treg cells, pro-/anti-inflammatory cytokines, and M1/M2 macrophages). We compared protein expression levels between two classes of samples based on the normalized HNSCC protein expression profiles dataset in TCGA using Student’s $t$-Test. The false discovery rate (FDR) was calculated by the Benjamini and Hochberg (BH) method [51] to obtain the adjusted $P$-values in multiple tests. The threshold of FDR < 0.05 indicates the statistical significance.

4.3. Gene-Set Enrichment Analysis

We performed gene-set enrichment analysis of the HNSCC gene expression profiling data by GSEA [26] and identified the KEGG [52] pathways that were differentially expressed between TP53-mutated and TP53-wildtype HNSCCs or between HRAS-mutated and HRAS-wildtype HNSCCs (FDR < 0.05).

4.4. Comparison of Immune Cell Infiltration Levels between Two Classes of Samples

We quantified the immune cell infiltration level of each HNSCC sample using the immune score evaluated by ESTIMATE [53]. The Mann-Whitney $U$ test was used to compare the immune scores between two groups of HNSCCs.

4.5. Logistic Regression Model for Predicting Immune Signature Levels

To evaluate the contributions of different molecular features in predicting immune signature levels, we used the logistic regression model with multiple predictors (TP53 mutation, HRAS mutation, cell cycle score, apoptosis score, HPV infection status, TMB, and aneuploidy). TP53 mutation and HRAS mutation were binary variables (mutated or wildtype), cell cycle score and apoptosis score were continuous variables (ssGSEA scores of the gene sets in the cell cycle and apoptosis pathways), HPV infection status was a binary variable (HPV+ or HPV-), TMB (defined as the total somatic mutation count in tumor) was a discrete variable, and tumor aneuploidy (defined as the tumor ploidy score generated by ABSOLUTE [54]) was a continuous variable. The tumors with high (upper quarter) versus low (bottom quarter) immune signature scores were predicted.

4.6. Investigation of the Correlation Between Pathway or Protein Activity and Immune Signatures in HNSCC

We investigated the correlation between p53- or RAS-mediated pathways and immune signatures in HNSCC. The ssGSEA score [49,50] was used to quantify the activity of a pathway in an HNSCC sample on the basis of the set of genes included in the pathway. The first-order partial correlation [55] was used to assess the correlations between pathways and immune signatures in order to correct for the strong correlation between the p53 pathway and the p53-mediated pathways and between the RAS pathway and the RAS-mediated pathways by control of the p53 and RAS pathways, respectively. The significance of the correlation between a pathway and an immune signature was determined with the threshold of FDR < 0.05. The Spearman correlation test was used to evaluate the correlation between protein expression levels and immune signature enrichment levels.

4.7. Survival Analyses

We classified HNSCC patients into two different classes based on immune signature enrichment levels (higher-enrichment-level (ssGSEA scores > the third quartile) versus lower-enrichment-level (ssGSEA scores < the first quartile), or immune scores (higher-immune-score (immune scores > the third quartile) versus lower-immune-score (immune scores < the first quartile). We used Kaplan-Meier survival curves to exhibit the survival (OS or disease free survival (DFS)) differences and the log-rank test to evaluate the survival-time differences between two classes of patients with a significance threshold of $P < 0.05$.

5. Conclusions

The TP53 mutation inhibited tumor immunity while the HRAS mutation promoted tumor immunity in HNSCC. These findings have potential clinical implications that the TP53 mutation and the HRAS mutation status could be useful biomarkers for identifying HNSCC patients responsive to immunotherapy.

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Ethics Approval and Consent to Participate

Ethical approval and consent to participate was waived since we used only publicly available data and materials in this study.

Consent for Publication

Not applicable.

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Authors’ Contributions

HL performed data analyses and helped prepare for the manuscript. ML performed data analyses and helped prepare for the manuscript. ZJ performed data analyses. ZL performed data analyses. XW conceived the research, designed analysis strategies, and wrote the manuscript. All the authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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