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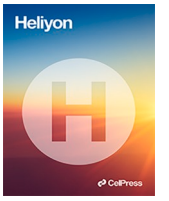

Research article

Overexpressed Wnt-7a acts as a potential antitumor immune modulator and predicts poor prognosis in HNSCC

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ABSTRACT

Immune checkpoint inhibitor (ICI) has become the first-line treatment of advanced head and neck squamous cell carcinoma (HNSCC), which has a relatively poor clinical prognosis and high mortality. However, only a proportion of patients respond well to ICI therapy. Identifying reliable predictive markers and potential targets to enhance the efficacy of ICI is of great importance. In this study, we systematically screened potential genes related to poor prognosis in HNSCC and Wnt-7a was selected based on results from multiple databases. Wnt-7a was overexpressed in HNSCC tissues and was associated with shorter overall survival. Human tissue samples and further bioinformatic analysis showed that Wnt-7a may be expressed in T cells and inhibited the infiltration and function of cytotoxic T cell response, both directly and indirectly through decrease the infiltration of Th1, Th17 cells and increasing the infiltration of Tregs. Genes that can directly interact with Wnt-7a were also identified. Surprisingly, 8 out of 9 genes identified were found to participate in the modulation of anti-tumor immunity. In addition, our study suggested that Wnt-7a, as a canonical Wnt ligand, could exert its function via regulating Wnt signaling and related mechanisms. Taken together, these results strongly support that Wnt-7a is a strong predictor of prognosis and ICI efficacy, and could be a promising potential target for enhancing the therapy response.

1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is a set of tumors that mainly derive from mucosal epithelium in the oral cavity, pharynx and larynx [1]. Most patients with HNSCC are diagnosed in the late stage, with limited therapeutic options. Recently, immune checkpoint inhibitors (ICIs) have been extensively applied in the recurrent or metastatic HNSCC and has been approved as the first-line therapy in advanced HNSCC. Nonetheless, therapeutic response of immune therapy varies between different patients [2]. Thus, it is of great importance to identify prognostic factors that is involved in the modulation of immune microenvironment and can predict the therapeutic responses, which may also provide new therapeutic targets in HNSCC.

Tumor microenvironment (TME) is rather complex and contains several critical cellular types that may influence the therapeutic effect of ICIs including immune cells and cancer associated fibroblast (CAFs), *etc.* [3]. HNSCC tissues are generally considered as "cold

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tumor" [4]. Nonetheless, infiltrating immune cells including Regulatory T cells (Tregs), $CD8^+$ tumor-infiltrating lymphocytes (TILs), T helper 17 (Th17) and T cytotoxic 17 (Tc17) cells in peripheral blood are all reported to be significant predictors of prognosis for patients with HNSCC [5–7]. Identifying molecular that are related to immune cell infiltrating and remodeling of antitumor immunity can be critical.

The function state of T cells is modulated by various factors. Of importance, the canonic Wnt signaling, an important stem cell-related signaling, plays vital role in potentiating the function of T cells. Canonical Wnt signaling pathway played an essential role for $CD8^+$ central memory T cell differentiation. Lack of TCF-1, a nuclear effector of the canonical Wnt signaling, impaired the ability of $CD8^+$ T cells to expand upon secondary challenge [8]. However, controversial result was found that Wnt signaling inhibited memory cytotoxic T lymphocyte (CTL) programming driving by IL-12 [9]. And $CD8^+$ cell-derived Wnt ligands suppressed infection expand upon HIV infection [10]. These results highlight the importance of Wnt signaling in regulating the function of T cells and indicating a context-dependent manner of the mechanism underlying Wnt signaling.

Wnt-7a is a ligand for the canonical Wnt signaling and plays controversial roles in the progression of different kinds of tumors [11]. Previous study showed that Wnt-7a is upregulated in oral squamous cell carcinoma (OSCC), which is subtype of HNSCC, and mediated chemoresistance to cisplatin [12]. Importantly, previous studies reported that Wnt-7a played a unique role in immune modulation including inducing Th1-polarization [13] and the polarization of monocyte-derived macrophages [14]. Accordingly, Wnt-7a is identified to be associated with the infiltration of immune cells in lung cancer [15]. Wnt-7a is associated with poor prognosis and infiltration of antigen presenting cells in pancreatic cancer [16]. These data indicating that Wnt-7a play an important role in regulating the tumor immune microenvironment. However, there is no systemic analysis illustrates the prognostic value of Wnt-7a and its relationship with immune microenvironment in HNSCC.

In this study, we used public databases and analyzed the expression pattern of Wnt-7a and its prognostic values in HNSCC. Further analysis found that Wnt-7a is mainly expressed in T cells and highly involved in the modulation of $CD4^+$ Th cells and $CD8^+$ cytotoxic T cells in the immune microenvironment through regulating the Wnt associated signaling.

2. Materials and methods

2.1. Dataset collection

The RNA sequencing data of tumor and paired-normal tissue of HNSCC were downloaded from the TCGA database (https://portal.gdc.cancer.gov/). Corresponding clinical information were downloaded from the TCGA-HNSC dataset (https://portal.gdc.cancer.gov/projects/TCGA-HNSC).

2.2. Expression analysis

The tissue- and cell-level expression analysis of *WNT7A* was performed via the Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/) [17]. The protein expression analysis of Wnt-7a was performed using immunohistochemical images of Wnt-7a expression from the Human Protein Atlas (HPA) (http://www.proteinatlas.org/) and further confirmed in tissue samples chip containing 47 HNSCC tissues and 5 normal tissues, which is purchased from Shanghai Outdo Biotech Company (Ctl No: JS W-11-01). Association between *WNT7A* expression and different cell markers were performed by GEPIA. Relative expression levels of these cell markers in *WNT7A*-high and low expression HNSCC patients were determined using GAMOIP database [18].

Immunohistochemical staining of the paraffin-embedded tissue sections were performed following standard protocols. The tissue sections were incubated overnight with a rabbit polyclonal antibody to Wnt-7a (Abcam, Cat #ab100792, RRID: AB_10858110). The histochemistry score was determined by multiplying the stained intensity scores by the extent of positive cells. The stain intensity was scored as follows: 0: blank, 1: yellow, 2: dark yellow and 3: brown. The extent of positive cells was scored as follows: 0: 0–5%, 1: 6–25 %, 2: 26–50 %, 3: 51–75 %, and 4:76–100 %. All slides were independently analyzed by two experienced pathologists.

2.3. Survival analysis

LinkedOmics (http://www.linkedomics.org/) [19] was used to obtain a list of genes of survival significance in HNSCC. Kaplan-Meier survival analysis and cox regression was used to determine the association of *WNT7A* expression level with overall survival (OS) in HNSCC patients. The association of *WNT7A* expression with OS was analyzed using data from TCGA in GAMOIP database. Subgroup analyses of association of *WNT7A* expression and OS in HNSCC patients that were stratified by different clinical characteristics were performed using data from TCGA database.

2.4. Correlation between WNT7A and TME

The infiltration of immune cells as well as cancer-associated fibroblast was estimated by GAMOIP database using 3 methods including CIBERSORT, MCPcounter and EPIC. Different characteristics of TME including immunogenicity (tumor mutation burden, neoantigen loads and MANTIS score) and immune scores (stromal fraction, intratumor heterogeneity, proliferation, wound healing, lymphocyte infiltration signature score, IFN-gamma response, and TGF-β response were also analyzed between *WNT7A* high and low expression groups in HNSCC patients.

2.5. Mutational landscape analysis

Mutational landscape of *WNT7A* high and low expression group in HNSCC patients was analyzed by GAMOIP database and the top 30 mutated genes were listed.

2.6. Enrichment analysis and protein–protein interactions (PPI) network of Wnt-7a

Genes that are positively and negatively related to *WNT7A* expression were analyzed and obtained from LinkedOmics. The KEGG and GO analysis of top 500 genes that are positively and negatively related to *WNT7A* expression were performed using gene set enrichment analysis (GSEA) and presented using R package ggplot2.

The PPI network of Wnt-7a is constructed using the STRING database (http://string-db.org/) and hub genes of the PPI network were calculated and presented by Cytoscape 3.8.0 software.

2.7. Statistical analysis

GraphPad prism 8.0 was utilized for statistical analysis in our study. The expression difference of Wnt-7a in HNSCC and normal tissues determined by IHC score were analyzed by Dunn's multiple comparisons test. Mann-Whitney test (two groups comparison) and Tukey's multiple comparisons test (three group comparison) were performed to determine the potential correlation of *WNT7A* expression with clinicopathologic characteristics in TCGA HNSCC cohort. Log-rank test was performed to determine the association of

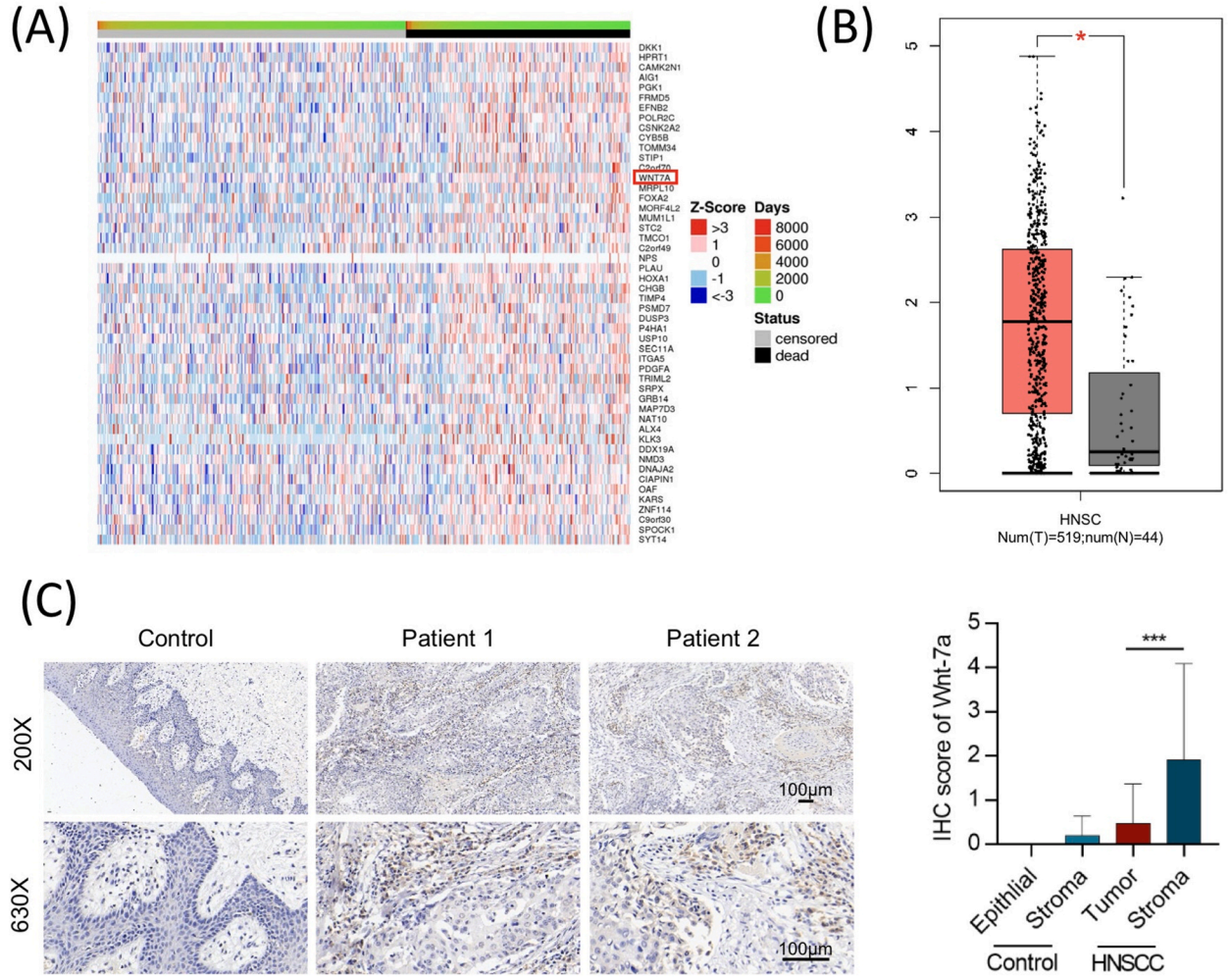

Fig. 1. Wnt-7a is overexpressed in HNSCC. (A) Heatmap showing the top 50 genes that are positively related to the prognosis of HNSCC. (B) The mRNA level of *WNT7A* in HNSCC tissues and controls ((TCGA and GTEx database). (C) Representative images of IHC staining of Wnt-7a and its IHC scores in HNSCC tissue and normal tissues. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

WNT7A expression and OS in TCGA HNSCC cohort and corresponding subgroups. A two-tailed $p < 0.05$ was considered as statistical significance.

3. Results

3.1. Wnt-7a is overexpressed in HNSCC tissues

To identify a prognostic marker and potential therapeutic target for HNSCC, we firstly used the LinkedOmics database [19] to obtain a list of genes that are negatively related with the prognosis of HNSCC patients (Fig. 1A and Supplementary Table 1). Notably, the role of Wnt-7a, which is a ligand for Wnt signaling [20] and reported in many other tumors [21–23], has not been illustrated in HNSCC. We further found in GEPIA database that *WNT7A* is overexpressed in HNSCC tissues compared to normal controls using data from TCGA database and GTEx data (Fig. 1B). In addition, *WNT7A* is also found highly expressed in several other tumors including cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), uterine corpus endometrial carcinoma (UCEC) and uterine carcinosarcoma (UCS), which may indicate an extensive tumor-promoting role of Wnt-7a (Supplementary Fig. 1).

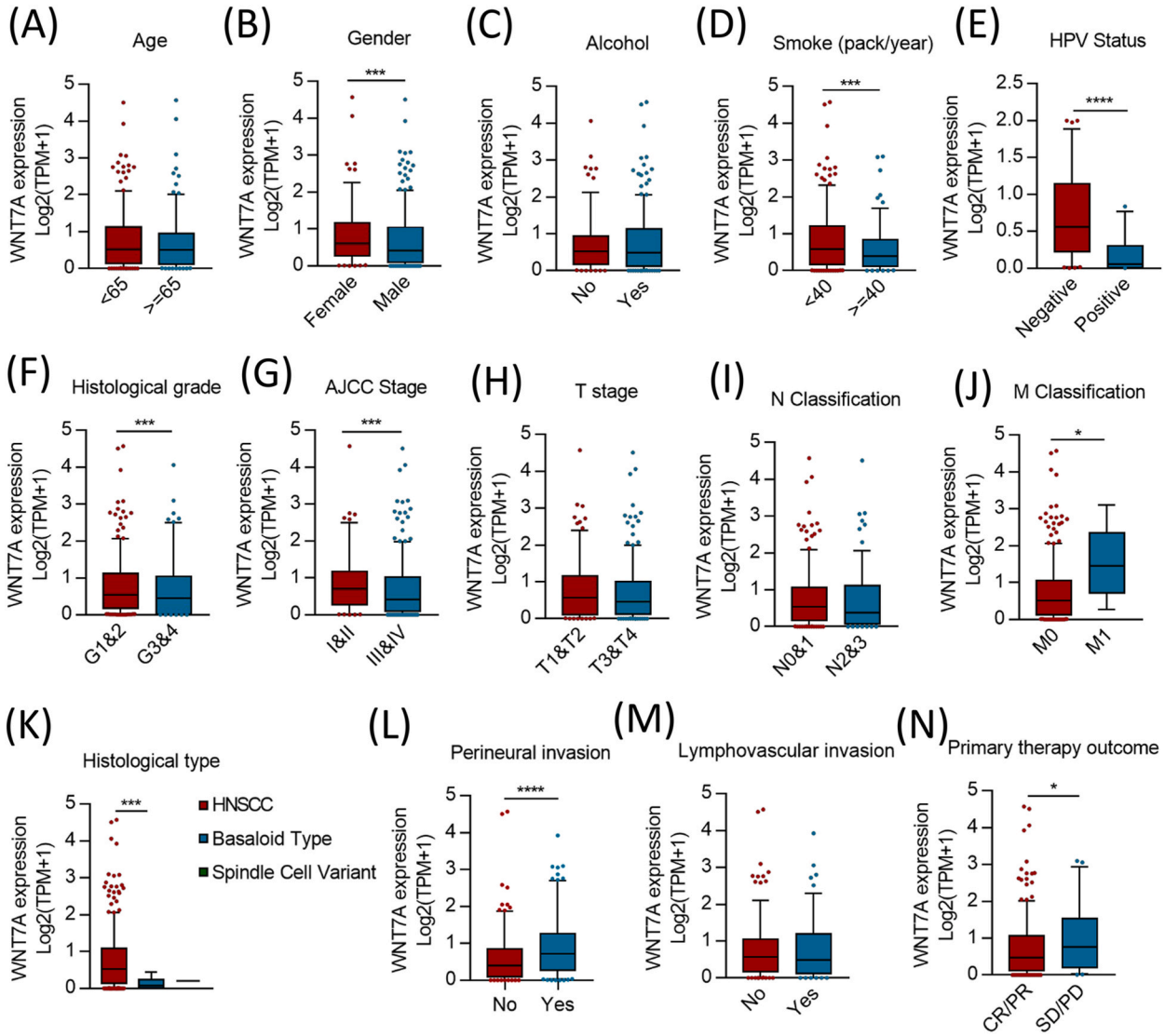

Fig. 2. Correlation analysis between expression of *WNT7A* and clinicopathologic parameters in TCGA-HNSCC patients. *WNT7A* expression in HNSCC samples with different age (A), gender (B), alcohol intake (C), smoke (D), HPV status (E), histological grade (F), AJCC stage (G), TNM stage (H–J), histological type (K), perineural invasion (L), lymphovascular invasion (M) and primary therapy outcome (N). (*$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$).

To further confirm the expression pattern of Wnt-7a, we applied a tissue chip that contains 47 HNSCC tissues and 5 normal control tissues. Constant with the existing data in HPA database, immunohistochemical staining showed that Wnt-7a is generally lowly expressed both in control and HNSCC tissues (Fig. 1C). Surprisingly, we found that Wnt-7a is relatively enriched in stroma cells than in tumor cells (Fig. 1C).

3.2. *Wnt-7a predicts poor prognosis in TCGA-HNSC patients*

To further clarify the role of Wnt-7a in HNSCC patients, we next analyzed the association between *WNT7A* expression and clinical features using data from TCGA database. We first analyzed the correlation between *WNT7A* expression and general information of

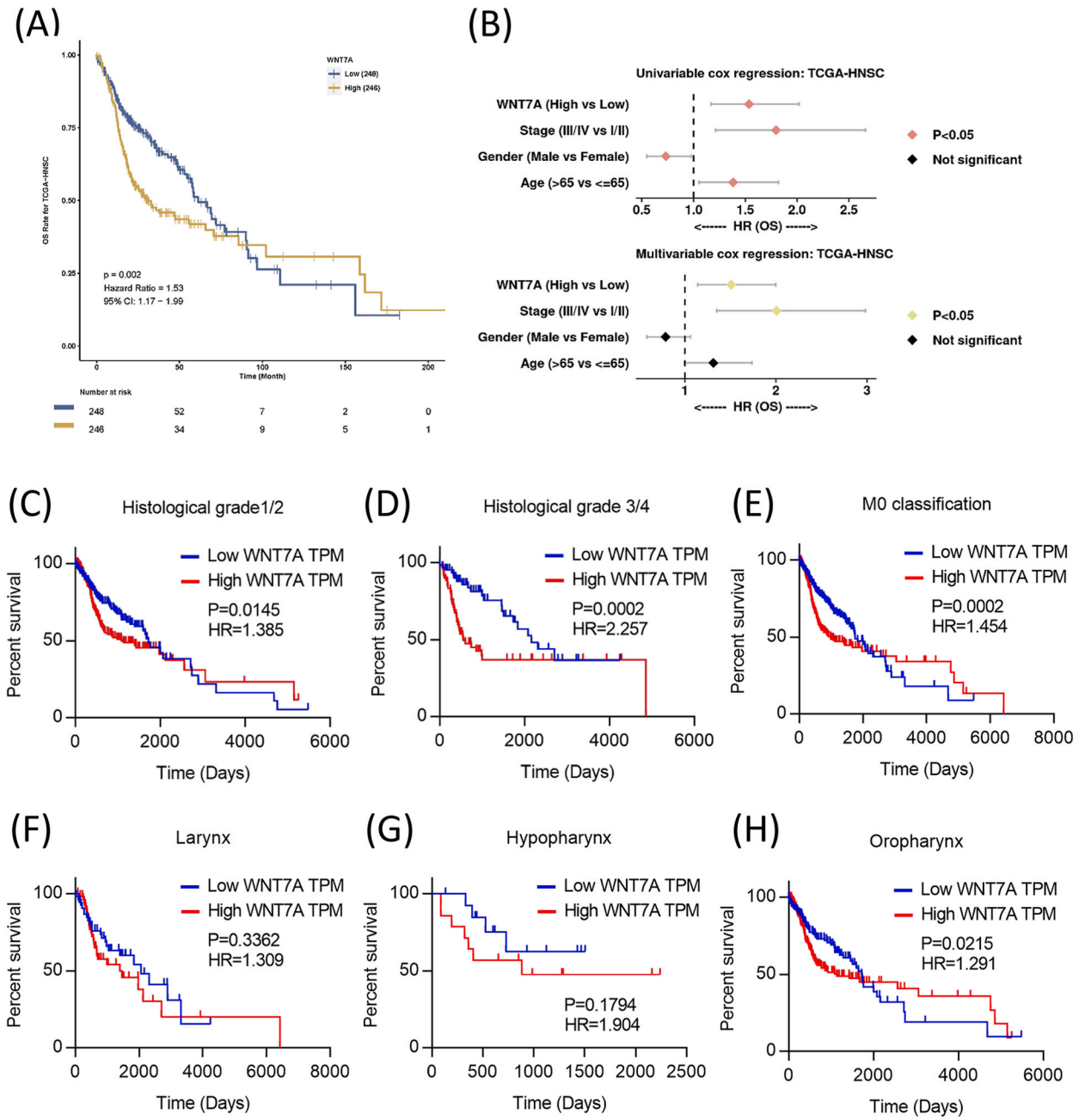

Fig. 3. Overexpressed *WNT7A* predicts poor prognosis in HNSCC patients. (A) Kaplan-Meier survival analysis showing association between *WNT7A* expression and OS. (B) Univariate and multivariate Cox regression analysis of OS in TCGA-HNSCC. Survival analysis of NT5E in HNSCC subgroups including different histological grades (C, D), M classification (E) and anatomic site (F–H).

HNSCC patients (Fig. 2A–E). The result indicated that there was no expression difference of *WNT7A* in patients with different age and alcohol intake status. Male HNSCC patients and patients who smoked more than 40 pack per year had a relatively lower level of *WNT7A* expression. In terms of tumor conditions, *WNT7A* expression level is higher in patients with lower AJCC stage, lower histologic grade (Fig. 2F and G). Notably, *WNT7A* is relatively enriched in patients with HPV negative status, metastasis, perineural invasion and

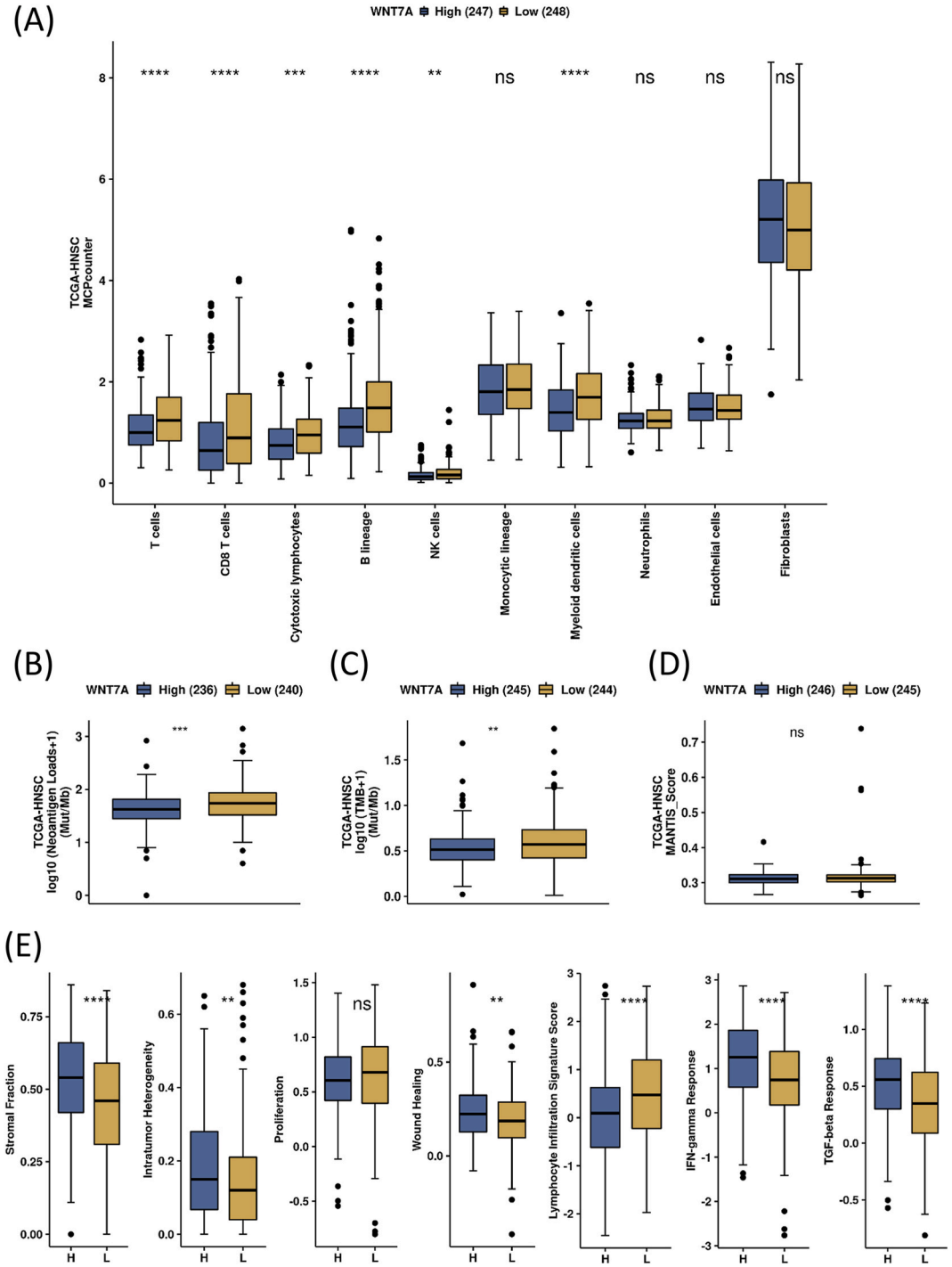

Fig. 4. *WNT7A* is associated with a unique tumor microenvironment in HNSCC. (A) Correlation between *WNT7A* expression and MCPcounter score of various infiltrated cells. Different neoantigen loads (B), tumor mutation burden (C) and MANTIS score (D) in *WNT7A* high and low expression HNSCC tissues. (E) Immune scores including stromal fraction, intratumor heterogeneity, proliferation, wound healing, lymphocyte infiltration signature score, IFN-gamma response, and TGF-β response in *WNT7A* high and low expression groups. (**$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

more importantly, patients with poor response to primary therapy (Fig. 2E–J, L, N), which all indicates poor prognosis [24–27]. In addition, expression of *WNT7A* is similar in patients with different T stage, N classification, and with/without lymphovascular invasion (Fig. 2H, I, M). Although a statistical difference was found in patients with different histological types, the number of HNSCC patients with basaloid type is rather few to draw any solid conclusion (Fig. 2K).

Since *WNT7A* expression is associated with different clinical features that can be associated with both good and bad outcomes, we further investigated the prognostic value of *WNT7A* in HNSCC. Using GAMOIP database, we found that higher *WNT7A* expression predicted poorer outcomes including shorter OS (Fig. 3A and B). We further investigated whether *WNT7A* can predict OS in subgroups of HNSCC patients using data from TCGA database. The results indicated that *WNT7A* can predict poor outcomes in patients with different histologic grade and without metastasis (Fig. 3C–E). Notably, OS in patients of G3/G4 histologic grades with high *WNT7A* expression is significantly shorter (Fig. 3D). Moreover, *WNT7A* is associated with poor prognosis in oropharynx but not in larynx and hypopharynx (Fig. 3F–H), which may be because of limited number of samples in TCGA database. In general, high *WNT7A* expression level can predict poor prognosis in oral squamous cell carcinoma (OSCC) patients.

3.3. Overexpressed Wnt-7a is associated with a unique immune-suppressive microenvironment in HNSCC

Wnt signaling was previously reported to participate in immunosurveillance in various cancers [28]. To investigate the underlying mechanism of the tumor-promoting role of Wnt-7a in HNSCC, we first explored whether Wnt-7a can influence tumor immune microenvironment in HNSCC using GAMOIP database with three different methods including MCPcounter, CIBERSORT and EPIC. Interestingly, we found that *WNT7A* expression is negatively associated with $CD8^+$ T cell and natural killer (NK) cell infiltration, which are cytotoxic cells and exert anti-tumor functions in various types of cancer [29,30] (Fig. 4A, Supplementary Figs. 2A and B). In addition, B cell infiltration was found higher in *WNT7A*-low expression group using all 3 methods, and CAF infiltration was lower in *WNT7A*-low expression group using the EPIC method. Correspondingly, the immunogenicity of *WNT7A*-high expression group was also lower as indicated by tumor mutation burden and neoantigen loads, which may predict poor response to immune therapy (Fig. 4B and C). No significant difference was found when it comes to the MANTIS score (Fig. 4D). Immune score analysis was also conducted and the result showed that *WNT7A*-high expression group has more stromal fraction, intratumor heterogeneity, wound healing, IFN-gamma response, and TGF-β response, while lymphocyte infiltration signature score was significantly lower. Taken together, these data indicated that overexpressed *WNT7A* is associated with an immune-suppressive TME and may predict poor response to immune therapy.

Since our previous data indicated that Wnt-7a was mainly expressed in the stromal compartment of HNSCC tissues, we further determined *WNT7A* expression in cell level using GEPIA2021 database. Immune cells and CAFs whose infiltration were associated with *WNT7A* expression as previously described were analyzed. Compared with control tissues, *WNT7A* expression in $CD4^+$ T cells was higher and lower in $CD8^+$ T cells in HNSCC tissues (Fig. 5A). No obvious *WNT7A* expression was found in B cell, NK cells and CAFs (Fig. 5A). We next further determined the correlation of *WNT7A* expression and cell markers of different types of $CD4^+$/$CD8^+$ T cells to specify the function of Wnt-7a in each cell type by both Pearson correlation analysis and directly analyzing the expression level of these markers in *WNT7A*-high/low expression groups. The results showed that *WNT7A* expression was negatively associated with gene signature of $CD8^+$ cytotoxic T cell, Th1 cell, Th2 cell and Th17 cell, and positively related with Treg signature [31], indicating an immune-suppressive role in HNSCC (Fig. 5B). Further analysis indicated that *WNT7A* expression was negatively correlated with many cytotoxic $CD8^+$ T cell markers including *CD8A*, *IFNG*, *GZMB*, *EOMES* and *ID2* (Fig. 5C and D) [29]. Correlation of *WNT7A* with markers of different subtypes of $CD4^+$ T cells were also shown (Supplementary Fig. 3), and inconsistent with our previous result, no obvious correlation was found between *WNT7A* expression and Th2 cell markers. Notably, *WNT7A* expression was negatively correlated to exhausted T cell marker *TIGIT* and *PDCD1*(PD-1) (Fig. 5E and F) [32,33], also indicating a poor response to immune therapy in *WNT7A* high-expressed group.

3.4. Wnt-7a participates in cancer-promoting and modulating anti-tumor immunity molecular pathways

To further investigate the molecular mechanism of Wnt-7a, we divided HNSCC samples from TCGA database into *WNT7A* high expression and low expression group, analyzed the transcriptome sequencing data and performed GSEA. The results showed that stem cell signaling, such as Wnt and Notch signaling, and other cancer associated signaling such as pathways in cancer and ERBB2 signaling is enriched in *WNT7A* high expression group (Fig. 6A). Notably, multiple metabolic pathways were enriched in *WNT7A* low expression group (Fig. 6B).

To specify which pathway or molecular that is directly regulated by *WNT7A* in HNSCC, we analyzed transcriptome sequencing data of HNSCC patients from TCGA database. 4605 genes were found positively correlated with *WNT7A* expression and 9157 genes were negatively related (Supplementary Table 2). The top 500 positively and negatively related genes were further applied for KEGG and GO pathway analysis. The result showed that pro-tumoral signaling pathways such as PI3K-AKT pathway and Rap1 pathway were enriched in genes that positively related to *WNT7A* expression. On the contrary, pathways that are mainly involved in anti-tumor biological process such as the p53 pathway is enriched in genes negatively related to *WNT7A* (Fig. 6C and D, Supplementary Fig. 4A). Using Cytoscape software, we constructed a PPI network to identify hub genes that are directly interacted with *WNT7A*. Only 9 genes were identified including 6 positively related and 3 negatively related genes (Fig. 6E). Of the 6 positively related genes, 5 of them are associated with Wnt signaling including *DKK3*, *PTK7*, *DVL1*, *LHX1* and *MSX2* [34,35]. Surprisingly, all of the 6 positively related genes were found negatively related with $CD8^+$ T cell infiltration using least 2 statistical methods in HNSCC (Fig. 6F, Supplementary Figs. 4B and C). And all of them are negatively associated with the infiltration of cytotoxic lymphocytes (Fig. 6F). Of the 3

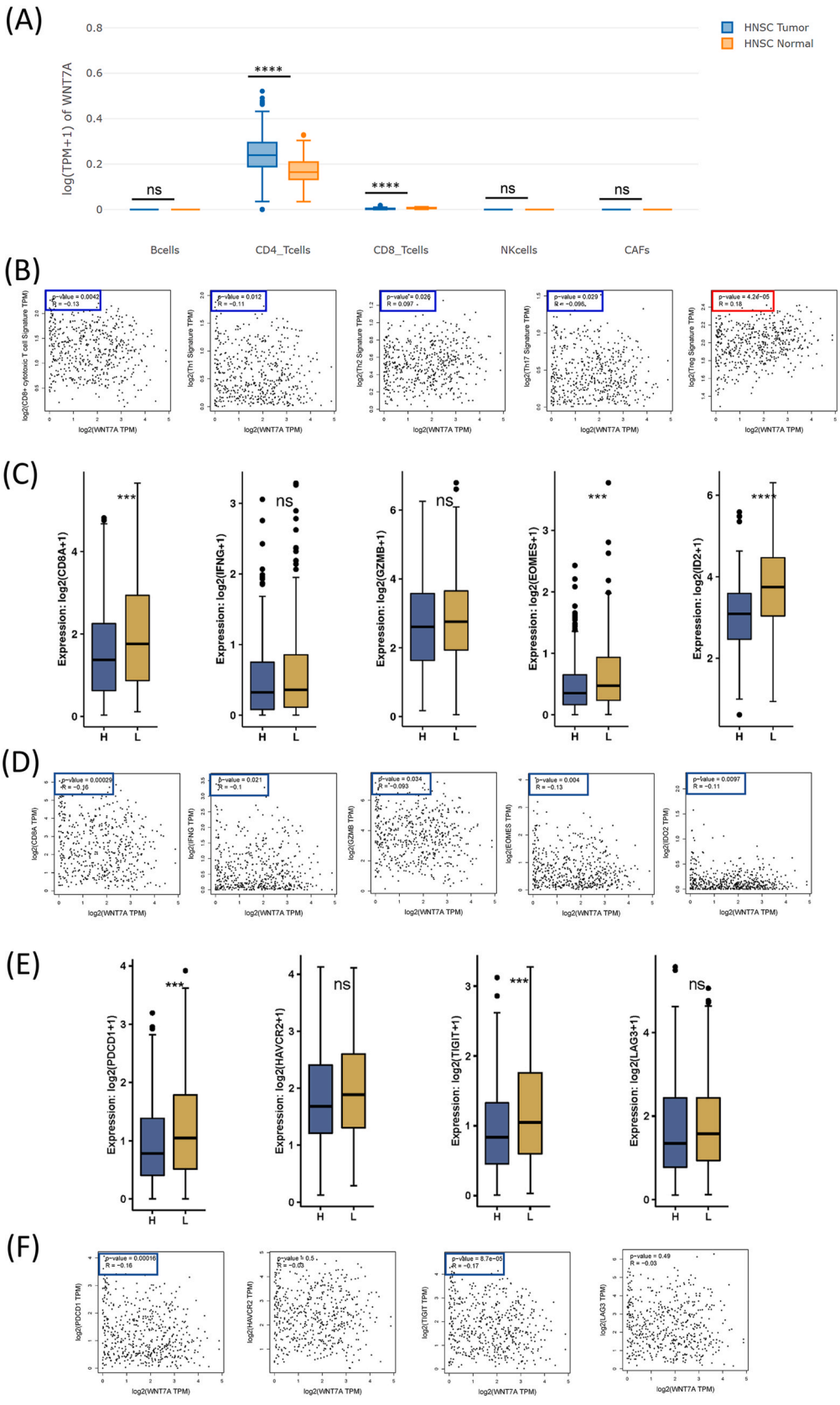

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Fig. 5. *WNT7A* is mainly expressed in T cells and inhibits antitumor immunity. (A) *WNT7A* expression patterns in infiltrated cell based on the GEPIA2021 database. (B) Correlation between *WNT7A* expression and gene signatures of $CD8^+$ cytotoxic T cells, Th1 cells, Th2 cells, Th17 cells and Tregs. (C) Comparison of $CD8^+$ cytotoxic T cell-related markers between *WNT7A* high and low expressed groups. (D) Correlation between *WNT7A* expression and $CD8^+$ cytotoxic T cell-related markers. (E) Comparison of exhausted T cell-related markers between *WNT7A* high and low expressed groups. (F) Correlation between *WNT7A* expression and exhausted T cell-related markers. (***$p < 0.001$, ****$p < 0.0001$).

negatively related genes, *BCL2* and *SOX2* was found positively related to $CD8^+$ T cell infiltration, and no correlation was found in *PTCH1* (Fig. 6F, Supplementary Figs. 4B and C).

In addition, we also analyzed the mutational difference between *WNT7A* high and low expression group. The result showed that the mutational subtypes of *P53*, *CDKN2A* and *APOB* were significantly different (Supplementary Fig. 5A). Further analysis showed that *WNT7A* expression is higher in *P53*, *APOB* mutated and lower in *CDKN2A* mutated HNSCC tissues than in wildtype (Supplementary Figs. 5B–D), indicating that *WNT7A* expression could be regulated by these genetic changes.

4. Discussion

Although patients with HNSCC have a relatively better overall prognosis than other tumors, HNSCC patients are often diagnosed at late stage, which has a 5-year survival rate of only 40 % [36]. Chemotherapy and radiation have limited therapeutic effect in HNSCC patients at late stage, while ICI has emerged as an effective therapy, but only a proportion of HNSCC patients respond to ICI. The underlying mechanism is yet remain uncovered and requires further exploration.

Wnt-7a plays controversial roles in different cancers. While Wnt-7a promotes the migration and invasion in gastric cancer [37], colorectal cancer [38], bladder cancer, *etc.*, it can inhibit the progression of non-small cell lung cancer [39] and hepatocellular carcinoma [40], *etc*. Here in this study, we found that overexpressed Wnt-7a is associated with poor prognosis in HNSCC. Notably, we found that Wnt-7a is mainly expressed in $CD4^+$ and $CD8^+$ T cells in HNSCC tissues and remodeled the immune microenvironment through regulating the anti-tumor immunity.

Direct cytotoxic effect of $CD8^+$ T cells are the main source of anti-tumor immunity, and T helper cells including Th1, Th2, Th17 cells are required for the antigen presenting, activation and enhancing of $CD8^+$ cytotoxic T cell functions [31]. T cell exhaustion has been recognized in tumors and is found associated with response to ICI therapy. The blockade of TIGIT/CD155 signaling was able to reverse T cell exhaustion and enhance anti-tumor immunity [41]. Our study illustrated that overexpressed Wnt-7a in HNSCC was associated with less anti-tumor immune cell infiltration including $CD8^+$ T cell and 3 subtypes of T helper cells including Th1, Th2, Th17 cells. Markers of cytotoxic effect of $CD8^+$ T cells were also negatively related to *WNT7A* expression. According to recent studies, the relationship of Wnt7a to immune infiltration in HNSCC is also present in other tumors. In pancreatic cancer, WNT7A was identified as genes that associated with poor prognosis and infiltration of antigen presenting cells [16]. Similar connection was also found in lung cancer [15]. In lung adenocarcinoma, it is found that norepinephrine inhibits $CD8^+$ T-cell infiltration and function largely through WNT7A/β-catenin signaling [42]. Further blocking WNT7A enhanced the infiltration and functionality of $CD8^+$ T cells, which bolstered antitumor immunity and improved the effectiveness of immune checkpoint blockade therapy [43]. These data indicate that Wnt-7a plays crucial roles in regulating the anti-tumor immunity, and could be a potential therapeutic target for HNSCC.

Wnt-7a can exert its function through or independent of activating the Wnt signaling. We further identified genes that directly related and interacted with Wnt-7a using data from TCGA database and found that Wnt-7a exert its function through activating Wnt associated signaling in HNSCC and inhibit the infiltration and function of $CD8^+$ T cells. Of the 6 genes that were positively regulated by Wnt-7a, *DKK3*, *JAG1*, and *DVL1* were all reported to either directly inhibit the infiltration and function of $CD8^+$ T cell [44–46], or promoted $CD8^+$ T-cell tolerance [47]. On the contrary, Wnt-7a negatively regulated genes were reported to promote the function of $CD8^+$ T cell. To specify, Sox2 caused immune evasion of $CD8^+$ T cell killing through alleviating the JAK-STAT [48], and Bcl2 were reported to increase the infiltration of $CD8^+$ T cell and prevent the apoptosis of CTLs in multiple cancer types [49–52]. These data further strengthened our hypothesis that overexpressed Wnt-7a in HNSCC is responsible for inhibited anti-tumor immunity and therefore poor prognosis. In addition, it is recently reported that Wnt-7a could promote tumorigenesis of HNSCC via activating FZD7/JAK1/STAT3 signaling instead of the Wnt signaling [53]. And stromal fibroblast-induced Wnt-7a could promote cancer cell migration via the AKT/CLDN1 signaling in OSCC [54], indicating the multiple potential roles and mechanisms of Wnt-7a.

In conclusion, we found that Wnt-7a acts as a prognostic factor that is associated with poorer prognosis in HNSCC through inhibiting the infiltration and cytotoxic function of $CD8^+$ T cells probably through modulating Wnt signaling. Our results provide a new potential biomarker for predicting the therapeutic response of HNSCC patients and a promising target for improving the therapeutic effect of ICIs.

CRediT authorship contribution statement

Hui-chao Jiang: Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ya Gao:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Shu-yan Wang:** Formal analysis, Data curation. **Yong-lan Zhao:** Methodology, Conceptualization. **Hai-peng Sun:** Writing – review & editing, Supervision, Resources, Methodology.

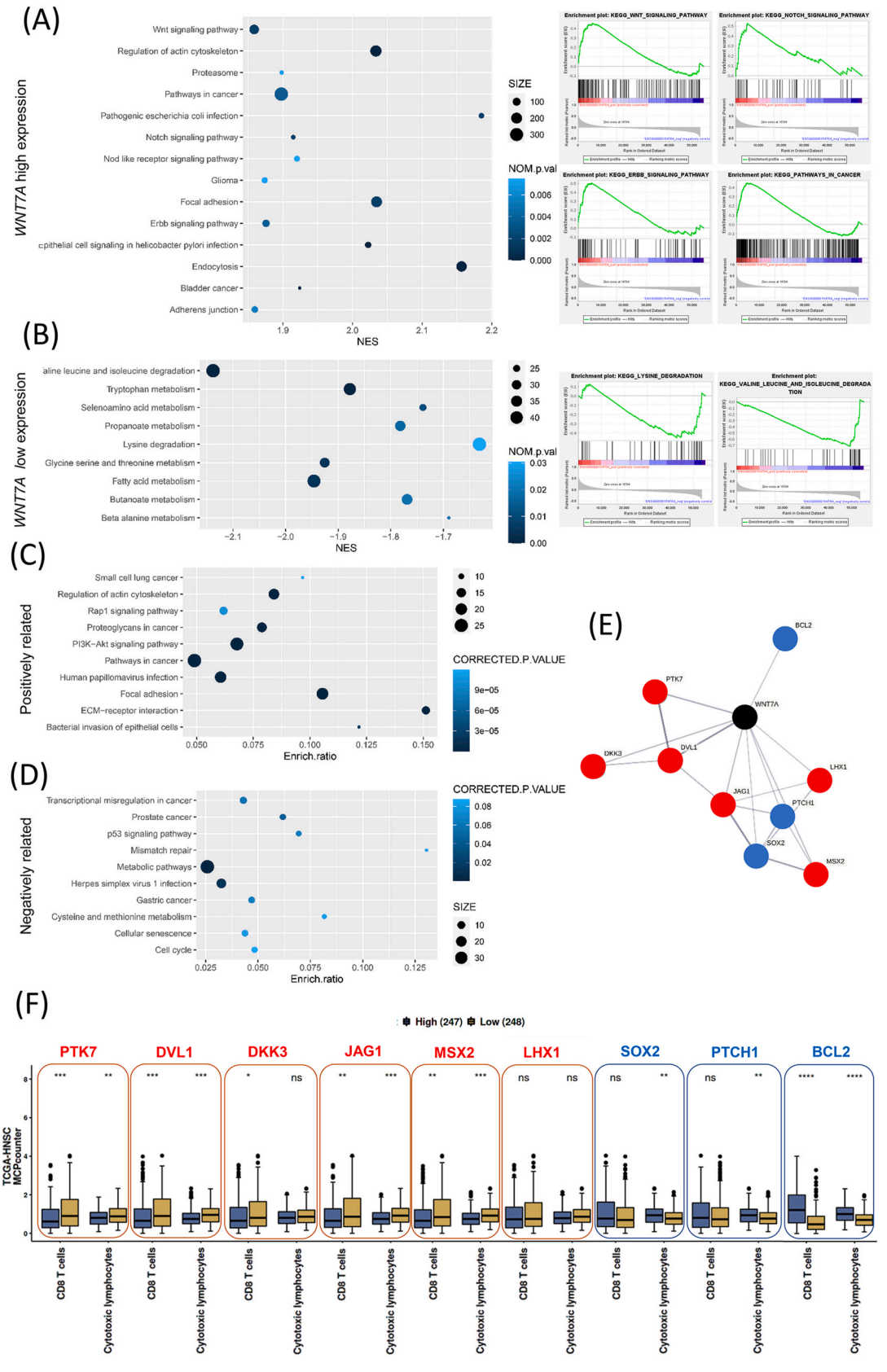

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Fig. 6. Potential mechanisms of Wnt-7a function in HNSCC. (A) GSEA and KEGG analysis of *WNT7A* high (A) and low (B) expression groups in HNSCC. GSEA and KEGG analysis of genes positively (C) and negatively (D) related to *WNT7A* expression in HNSCC. (E) PPI network showing hub genes of *WNT7A* in HNSCC, red ones are positively related to *WNT7A* and blue ones are negatively related. (F) Correlation between hub genes of Wnt-7a presented in (E) and MCPcounter score of infiltrated CD8 T cells and cytotoxic lymphocytes. (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2025.e42794.

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