The value of *WNT5A* as prognostic and immunological biomarker in pan-cancer

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Background: Finding new immune-related biomarkers is one of the promising research directions for tumor immunotherapy. The *WNT5A* gene could stimulate the WNT pathway and regulate the progression of various tumors. Recent studies have partially revealed the relationship between *WNT5A* and tumor immunity, but the correlation and underlying mechanisms in pan-cancer remain obscure. Thus, we conducted this study aiming to characterize the prognostic value and immunological portrait of *WNT5A* in cancer.

Methods: The data obtained from The Cancer Genome Atlas (TCGA), Genotype-Tissue Expression (GTEx), and Cancer Cell Line Encyclopedia (CCLE) databases was utilized to analyze *WNT5A* expression levels by Kruskal-Wallis test and correlation to prognosis by Cox regression test and Kaplan-Meier test, while the data was also used to study the association between *WNT5A* expression and immune microenvironment, immune neoantigens, immune checkpoints, tumor mutational burden (TMB), and microsatellite instability (MSI) in pan-cancer. Gene set enrichment analysis (GSEA) was used to clarify the relevant signaling pathways. The R package was used for data analysis and to create the plots.

Results: The pan-cancer analysis revealed that the expression level of *WNT5A* is generally elevated in most tumors (19/34, 55.88%), and high *WNT5A* expression was correlated with poor prognosis in esophageal carcinoma (ESCA, $P<0.05$), low-grade glioma (LGG, $P<0.01$), adrenocortical carcinoma (ACC, $P<0.01$), pancreatic adenocarcinoma (PAAD, $P<0.01$), and head and neck squamous cell carcinoma (HNSC, $P<0.05$). In addition, *WNT5A* expression was positively associated with immune infiltration, stromal score, and immune checkpoints in most cancers, and correlated to immune neoantigens, TMB, and MSI. Finally, GSEA indicated that *WNT5A* is implicated in the transforming growth factor β (TGFβ), Notch, and Hedgehog signaling pathways, which may be related to tumor immunity.

Conclusions: The expression of *WNT5A* is elevated in most tumors and associated with tumor prognosis. Furthermore, *WNT5A* is associated with tumor immunity and may be an immunological biomarker in cancer.

Keywords: *WNT5A*; pan-cancer analysis; prognosis; immunity

Submitted Feb 18, 2022. Accepted for publication Apr 13, 2022.
doi: 10.21037/atm-22-1317
View this article at: https://dx.doi.org/10.21037/atm-22-1317

Introduction

Cancer is a widespread disease and is the leading cause of death worldwide (1). Despite the rapid development of various treatment approaches for cancers in recent years, prognosis, especially in advanced cancers, remains poor (2,3). Excitingly, the advent of immunotherapy has revolutionized the clinical practice of oncology. At present, the expression level of PD-L1 in tumor cells and tumor mutational burden (TMB) are commonly used as biomarkers. However, how to successfully identify patients benefitting from immunotherapy is still the major challenge for clinicians. Hence, seeking novel targets and prognostic biomarkers, especially those related to immunotherapy, is of profound significance. With the improvement of R package (https://www.r-project.org/; The R Foundation for Statistical Computing, Vienna, Austria) and public databases such as The Cancer Genome Atlas (TCGA), more and more therapeutic targets of cancer are being discovered by performing pan-cancer expression analysis through bioinformatic analysis (4).

The *WNT* proteins are a large family of secreted glycoprotein signaling molecules rich in cysteine which play an important role in tumor progression, including proliferation, differentiation, apoptosis, and migration (5). At least 19 members of the *WNT* family have been identified and divided into 2 types: classical *WNT*/β-catenin signal molecules and non-classical signal molecules, according to their different biological functions (5,6). The *WNT5A* gene belongs to non-classical signaling molecules binding to different receptor complexes, and although its role in tumorigenesis is generally considered to be carcinogenic activities, controversy exists regarding its specific role (7). Several studies have reported that *WNT5A* has carcinogenic effects in lung cancer (8), gastric cancer (9), breast cancer (10), melanoma (11), and pancreatic cancer (12). But it has shown tumor suppressive effects in colon cancer (13), neuroblastoma (14), and thyroid cancer (15). Furthermore, conflicting effects have been recorded in the same tumor type. For example, Wu *et al.* found that *WNT5A* was highly expressed and has a carcinogenic effect in invasive esophageal squamous cell carcinoma (ESCC) (16). However, Li *et al.* reported that *WNT5A* is often silenced by promoter methylation and shows tumor inhibition characteristics in ESCC (17). Therefore, the role of *WNT5A* in cancer needs to be further elucidated and systematic bioinformatics analysis of *WNT5A* in pan-cancer is the preferred option.

To date, immune checkpoint blockade therapy has altered the treatment scheme of various tumors (18). However, the low response rate in some tumor types is mainly due to the highly immunosuppressive microenvironment and the absence of T cell infiltration, which is an urgent problem to be solved in immunotherapy (19). In addition, accumulating evidence has revealed a novel role of *WNT5A* in immunomodulation. The evidence suggests that *WNT5A* has a double effect on the tumor microenvironment. On one side, it can activate the ROR1/Akt/p65 pathway to promote inflammation and chemotaxis of immune cells (19,20); on the other side, it can activate TLR/MyD88/p50 to promote the synthesis of the anti-inflammatory cytokine interleukin 10 (IL-10) and immune tolerance (19,21). More importantly, inhibition of *WNT5A* signaling has been shown to increase the expression of programmed death-ligand 1 (PD-L1) in tumor tissues, and enhance the activity of anti-programmed cell death protein 1 (PD-1) and anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) antibodies, improving the response to checkpoint inhibitor therapy (22,23). For these reasons, it is of great significance to provide insight into the relationship of *WNT5A* and tumor immunity. We present the following article in accordance with the REMARK reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-1317/rc).

Methods

In this study, we revealed the expression of WNT5A and its potential prognostic value in pan-cancer using TCGA, Genotype-Tissue Expression (GTEx), and Cancer Cell Line Encyclopedia (CCLE) datasets. We then performed correlation analysis between WNT5A expression level and immune checkpoints, tumor-infiltrating immune cells, TMB, and microsatellite instability (MSI), which are closely

Table 1 Abbreviations of the tumors

Abbreviations	Tumor name
ACC	Adrenocortical carcinoma
BLCA	Bladder cancer
BRCA	Breast cancer
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL	Cholangiocarcinoma
COAD	Colon adenocarcinoma
COADREAD	Colon and rectal cancer
DLBC	Lymphoid neoplasm diffuse large B-cell lymphoma
ESCA	Esophageal carcinoma
GBM	Glioblastoma multiforme
GBMLGG	Glioblastoma multiforme low-grade glioma
HNSC	Head and neck squamous cell carcinoma
KICH	Kidney chromophobe
KIPAN	Pan-kidney cohort (KICH+KIRC+KIRP)
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
LAML	Acute myeloid leukemia
LGG	Lower grade glioma
LIHC	Liver hepatocellular carcinoma
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
MESO	Mesothelioma
OV	Ovarian cancer
PAAD	Pancreatic adenocarcinoma
PCPG	Pheochromocytoma and paraganglioma
PRAD	Prostate adenocarcinoma
READ	Rectum adenocarcinoma
SARC	Sarcoma
STAD	Stomach adenocarcinoma
SKCM	Skin cutaneous melanoma
STES	Stomach and esophageal carcinoma
TGCT	Testicular germ cell tumors
THCA	Thyroid carcinoma
THYM	Thymoma
UCEC	Uterine corpus endometrial carcinoma
UCS	Uterine carcinosarcoma
UVM	Uveal melanoma
OS	Osteosarcoma
ALL	Acute lymphoblastic leukemia
NB	Neuroblastoma
WT	High-risk Wilms tumor

related to immunotherapy. Finally, we performed gene set enrichment analysis (GSEA) to identify the signaling pathways linked to WNT5A. Taken together, our pan-cancer analyses provide insights into the prognostic and immunotherapy role of WNT5A in various cancers.

Data acquisition

We downloaded *WNT5A* expression data of tumor and normal samples coupled with clinical information from TCGA (https://portal.gdc.cancer.gov) and GTEx dataset (https://commonfund.nih.gov/GTEx/). The *WNT5A* expression data of tumor cell lines were obtained from CCLE dataset (https://portals.broadinstitute.org/ccle). Moreover, cancer immune infiltration scores were analyzed with data from the Tumor Immune Estimation Resource (TIMER) database. The R package was used to analyze the data. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The full name and abbreviation of all the tumors are listed in *Table 1*.

Analysis of WNT5A expression levels

Kruskal-Wallis test line analysis of the *WNT5A* expression data was conducted to compare *WNT5A* messenger RNA (mRNA) expression in 31 different normal tissues and 21 various cancer cell lines. Then, the *WNT5A* expression levels compared between cancer and normal samples were evaluated with data solely from TCGA database. In addition, considering the small size of non-cancerous tissues in TCGA, the *WNT5A* expression data of the GTEx and TCGA databases was further analyzed.

Correlation analysis of WNT5A expression level and prognosis in pan-cancer

Survival analysis of the expression and survival data obtained from TCGA in pan-cancer was conducted to confirm the prognostic role of *WNT5A* in pan-cancer. For the predictive analysis, a one-way Cox regression test was used to reveal the correlation between *WNT5A* expression and patient survival. Furthermore, the Kaplan-Meier (K-M) test was used to analyze patient survival. Prognostic indicators consisted of overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI). The results were presented in the form of forest plots (Cox regression test) and survival curves (K-M test).

Correlation analysis of the role of WNT5A in immune infiltration and tumor microenvironment

To evaluate the performance of *WNT5A* in immune infiltration, Spearman's rank correlation coefficient was utilized to distinguish the role of *WNT5A* in immune cell infiltration, including B cells, $CD4^+$ T cells, $CD8^+$ T cells, macrophages, neutrophils, and dendritic cells (DCs). Furthermore, we implemented an Estimation of Stromal and Immune Cells in Malignant Tumors Using Expression data (ESTIMATE) algorithm to assess the tumor microenvironment-related scores obtained from the above mentioned databases.

Correlation analysis of WNT5A expression level and immune checkpoints and neoantigens

To further clarify the correlation between *WNT5A* and tumor immune activity, immune checkpoints and neoantigens were analyzed. Spearman's rank correlation coefficient was performed to analyze the relationship between the expression *of WNT5A* and immune checkpoints, which were segregated into inhibitory and stimulatory groups. In addition, the number of neoantigens in every sample was detected and counted using a scanner, and the analysis mentioned above was applied to evaluate the correlation of *WNT5A* expression and the neoantigens number.

Correlation analysis of WNT5A expression level and TMB and MSI

The TMB is a quantifiable biomarker reflecting the mutational number of a tumor cell; MSI refers to the occurrence of a new microsatellite allele phenomenon compared with normal tissue (24). Correlation of *WNT5A* expression with TMB and MSI was analyzed utilizing Pearson's correlation coefficient. Bubble charts were used to present the results.

GSEA

It is common for GSEA to be utilized to analyze and explain changes in the level of coordination pathways (25). The signaling pathway of *WNT5A* was analyzed by GSEA analysis with the R package clusterProfiler. The Kyoto Encyclopedia of Genes and Genomes (KEGG database; KEGG; https://www.kegg.jp.) and hallmark gene sets from the Molecular Signature Database (MsigDB) were applied. Pathways with normalized enrichment score $|NES| > 1.5$, false discovery rate (FDR) <0.25, and $P<0.01$ were considered significantly enriched.

Statistical analysis

Statistical analysis methods were described in the above parts. A value of $P<0.05$ (two-side) was considered significant.

Results

WNT5A is highly expressed in most cancers

Data from the CCLE database, GTEx dataset, and TCGA database were analyzed to evaluate the *WNT5A* expression in normal and tumor tissues. Data from the GTEx dataset showed *WNT5A* was normally expressed in 31 normal tissues, with higher expression levels present in the bladder, uterus, and vagina, and lower expression levels in blood and bone marrow (*Figure 1A*). The CCLE analysis demonstrated that *WNT5A* is more highly expressed in bone, soft tissue, and the thyroid, while more lowly expressed in biliary tract, intestine, pancreas, and stomach (*Figure 1B*). In order to explore the expression level of *WNT5A* in tumor and matched normal tissues, we first analyzed the data from TCGA database separately (*Figure 1C*), and then analyzed the data from both TCGA and GTEx datasets. These results showed that *WNT5A* expression was elevated (19/34, 55.88%) in lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), glioblastoma multiforme low-grade glioma (GBMLGG), low-grade glioma (LGG), breast cancer (BRCA), stomach and esophageal carcinoma (STES), kidney renal papillary cell

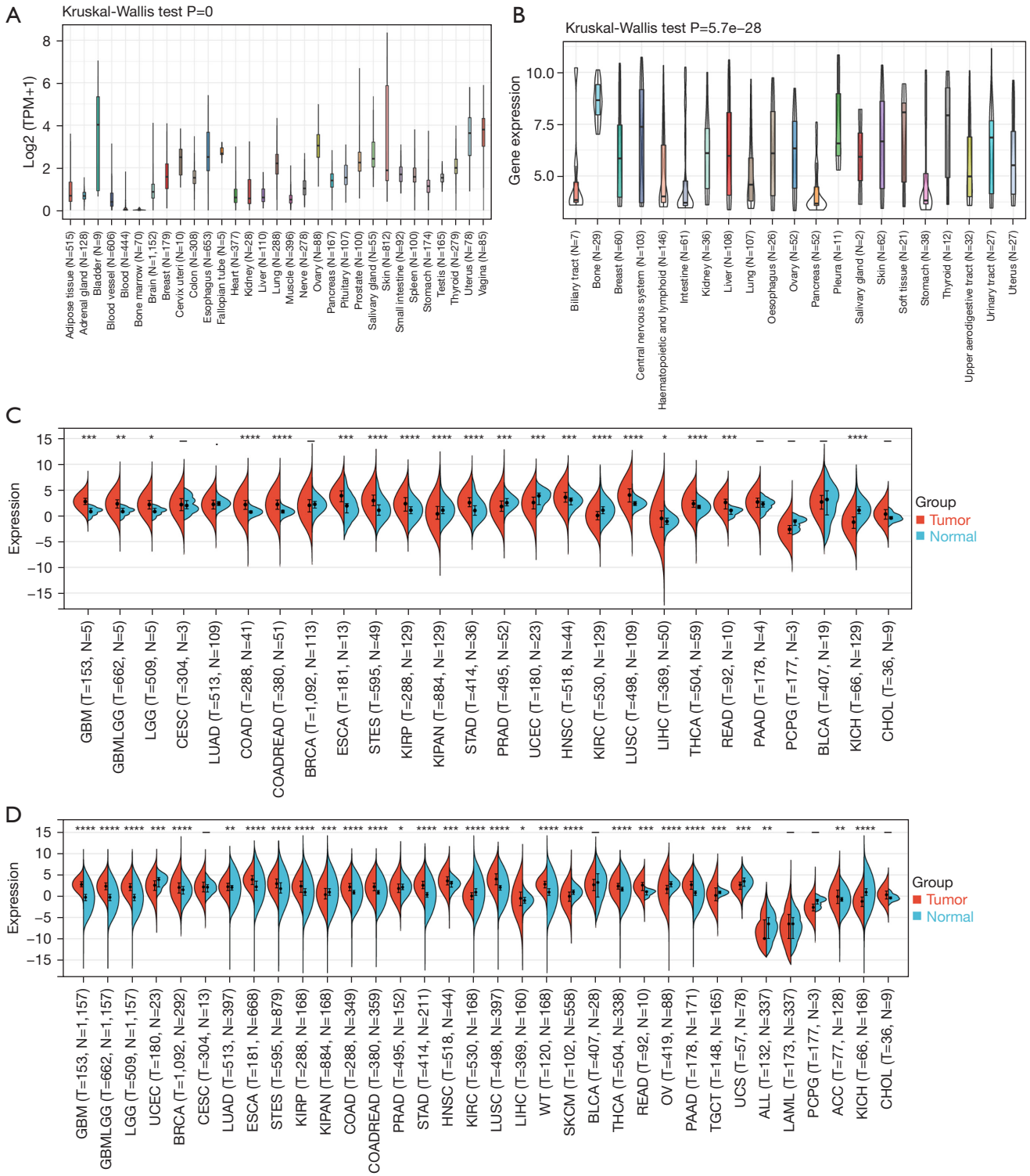

Figure 1 Expression levels of *WNT5A*. (A) *WNT5A* expression levels in normal tissues based on GTEx database. (B) *WNT5A* expression levels in tumor cell lines with data from CCLE database. (C) *WNT5A* expression levels in tumor and normal tissues using data from TCGA database. (D) *WNT5A* expression levels in tumor and normal tissues based on the consolidated data of GTEx and TCGA databases. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. GTEx, Genotype-Tissue Expression; CCLE, Cancer Cell Line Encyclopedia; TCGA, The Cancer Genome Atlas.

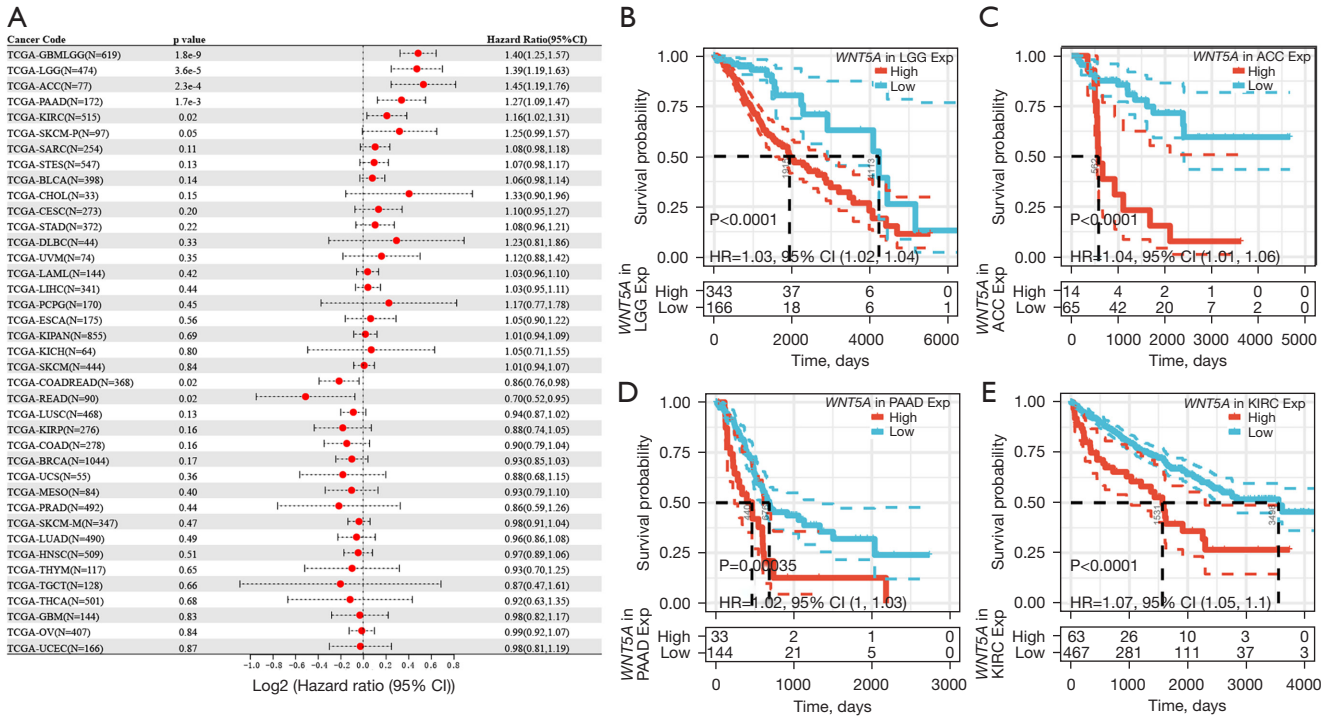

Figure 2 Associations between *WNT5A* expression and OS. (A) Cox analysis of *WNT5A* expression with OS in pan-cancer. (B-E) K-M analysis of *WNT5A* expression and OS in LGG, ACC, PAAD, and KIRC. OS, overall survival; K-M, Kaplan-Meier; LGG, low-grade glioma; ACC, adrenocortical carcinoma; PAAD, pancreatic adenocarcinoma; KIRC, kidney renal clear cell carcinoma.

carcinoma (KIRP), colon adenocarcinoma (COAD), colon and rectal cancer (COADREAD), stomach adenocarcinoma (STAD), head and neck squamous cell carcinoma (HNSC), liver hepatocellular carcinoma (LIHC), high-risk wilms tumor (WT), thyroid cancer (THCA), rectum adenocarcinoma (READ), pancreatic adenocarcinoma (PAAD), and adrenocortical carcinoma (ACC). However, *WNT5A* expression was lowered (10/34, 29.41%) in uterine corpus endometrial carcinoma (UCEC), KIPAN, prostate adenocarcinoma (PRAD), kidney renal clear cell carcinoma (KIRC), Skin cutaneous melanoma (SKCM), ovarian cancer (OV), testicular germ cell tumors (TGCT), uterine carcinosarcoma (UCS), acute lymphoblastic leukemia (ALL), and kidney chromophobe (KICH) (*Figure 1D*). These results revealed that the expression level of *WNT5A* is generally higher in the majority of tumors than that in corresponding normal tissues.

WNT5A is associated with prognosis in pan-cancer

To study the association between *WNT5A* expression and prognosis, we performed a survival association analysis for each cancer, including OS, DSS, DFI, and PFI. Cox proportional hazards model analysis showed that *WNT5A* expression levels were associated with OS in GBMLGG (HR =1.40, P<0.01), LGG (HR =1.39, P<0.01), ACC (HR =1.45, P<0.01), PAAD (HR =1.27, P<0.01), KIRC (HR =1.16, P=0.02), COADREAD (HR =0.86, P=0.02), and READ (HR =0.70, P=0.02) (*Figure 2A*). The K-M survival analysis revealed high expression of *WNT5A* was associated with poor OS in LGG (P<0.01, *Figure 2B*), ACC (P<0.01, *Figure 2C*), PAAD (P<0.01, *Figure 2D*), and KIRC (P<0.01, *Figure 2E*). In addition, Cox analysis results also revealed that *WNT5A* expression levels were associated with DSS in GBMLGG (HR =1.46, P<0.01), LGG (HR =1.44, P<0.01), ACC (HR =1.52, P<0.01), PAAD (HR =1.30, P<0.01), KIRP (HR =0.75, P<0.01), and LUSC (HR =0.86, P=0.01) (*Figure 3A*). Similarly, K-M survival analysis revealed that high expression of *WNT5A* was associated with poor DSS in LGG (P<0.01, *Figure 3B*), ACC (P<0.01, *Figure 3C*), PAAD (P<0.01, *Figure 3D*), and KIRC (P<0.01, *Figure 3E*), while higher expression level of *WNT5A* was associated with better DSS in KIRP (P<0.01, *Figure 3F*).

Moreover, regarding associations between *WNT5A*

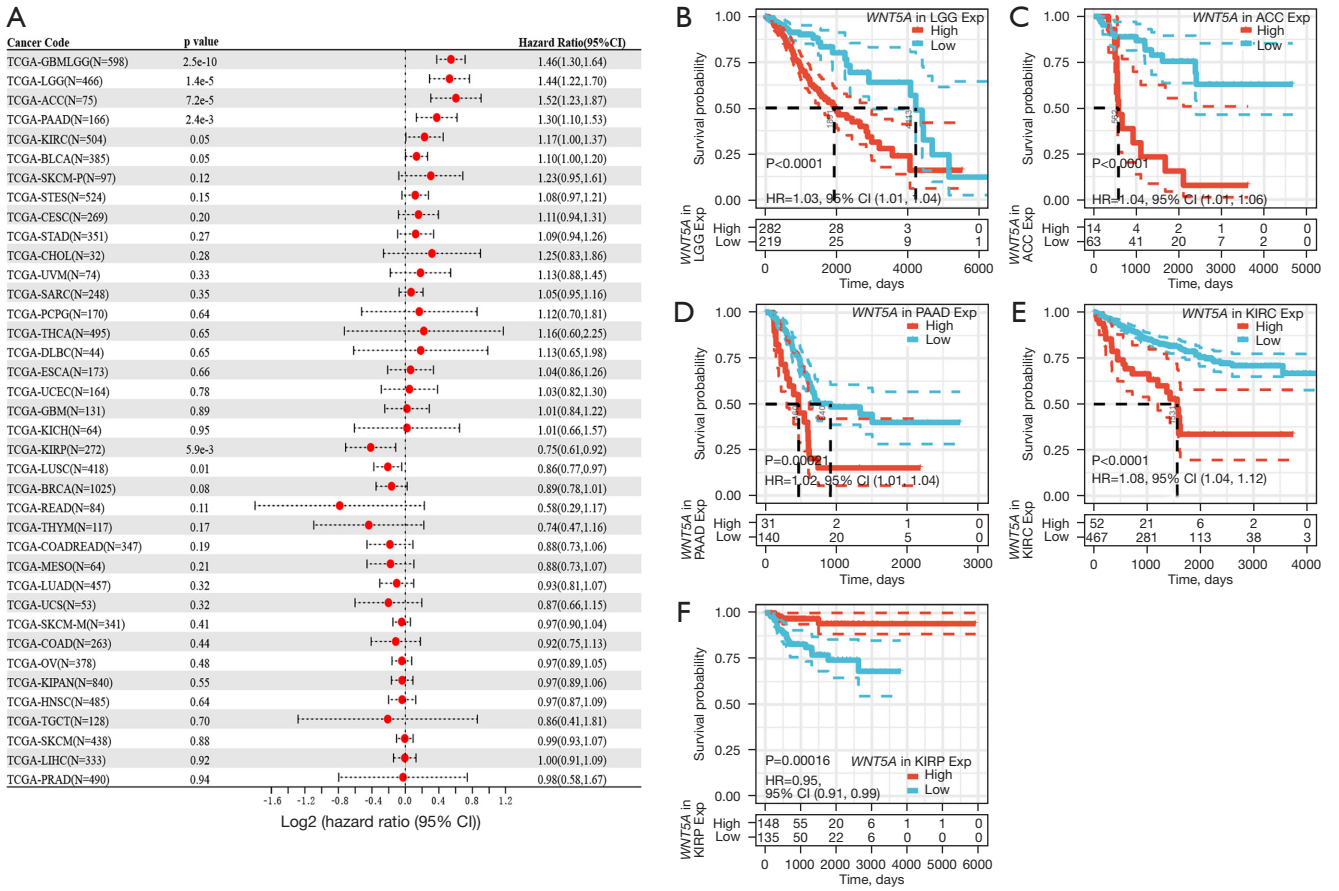

Figure 3 Associations between *WNT5A* expression and DSS. (A) Cox analysis of *WNT5A* expression with DSS in pan-cancer. (B-F) K-M analysis of *WNT5A* expression and DSS in LGG, ACC, PAAD, KIRC, and KIRP. DSS, disease-specific survival; K-M, Kaplan-Meier; LGG, low-grade glioma; ACC, adrenocortical carcinoma; PAAD, pancreatic adenocarcinoma; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma.

expression and DFI, Cox analysis depicted the relationship in PAAD (HR =2.49, P<0.01), COAD (HR =0.59, P<0.01), BRCA (HR =0.88, P=0.04), and COADREAD (HR =0.69, P=0.04) (*Figure 4A*). The K-M survival analysis revealed that high expression of *WNT5A* was associated with poor DFI in ESCA (P=0.021, *Figure 4B*), HNSC (P=0.03, *Figure 4C*), and PAAD (P<0.01, *Figure 4D*). Furthermore, Cox analysis found *WNT5A* expression was associated with PFI in GBMLGG (HR =1.33, P<0.01), LGG (HR =1.28, P<0.01), ACC (HR =1.35, P<0.01), KIRC (HR =1.21, P<0.01), PAAD (HR =1.21, P<0.01), STES (HR =1.11, P=0.02), bladder cancer (BLCA; HR =1.08, P=0.04), and KIRP (HR =0.85, P=0.03) (*Figure 5A*). The K-M survival analysis showed that high expression of *WNT5A* was associated with poor PFI in LGG (P<0.01, *Figure 5B*), ACC (P<0.01, *Figure 5C*), KIRC (P<0.01, *Figure 5D*), PAAD (P<0.01, *Figure 5E*), and PCPG (P=0.014, *Figure 5F*).

WNT5A affects tumor immune infiltration and microenvironment in pan-cancer

Tumor immune infiltration refers to the transfer of immune cells from blood to tumor tissues (26). To explore the role of *WNT5A* in tumor immunity, we first performed correlation analysis of *WNT5A* expression and various immune cells. Our data revealed the positive correlations between them in most cancers, especially in READ, PAAD, KIRC, LGG, PRAD, GBMLGG, THCA, PCPG, BRCA, COADREAD, and COAD, while negative correlations in TGCT and LUSC. But in thymoma (THYM), positive correlation with macrophages and negative correlation with $CD4^+$ T cells, $CD8^+$ T cells, neutrophils, and DCs

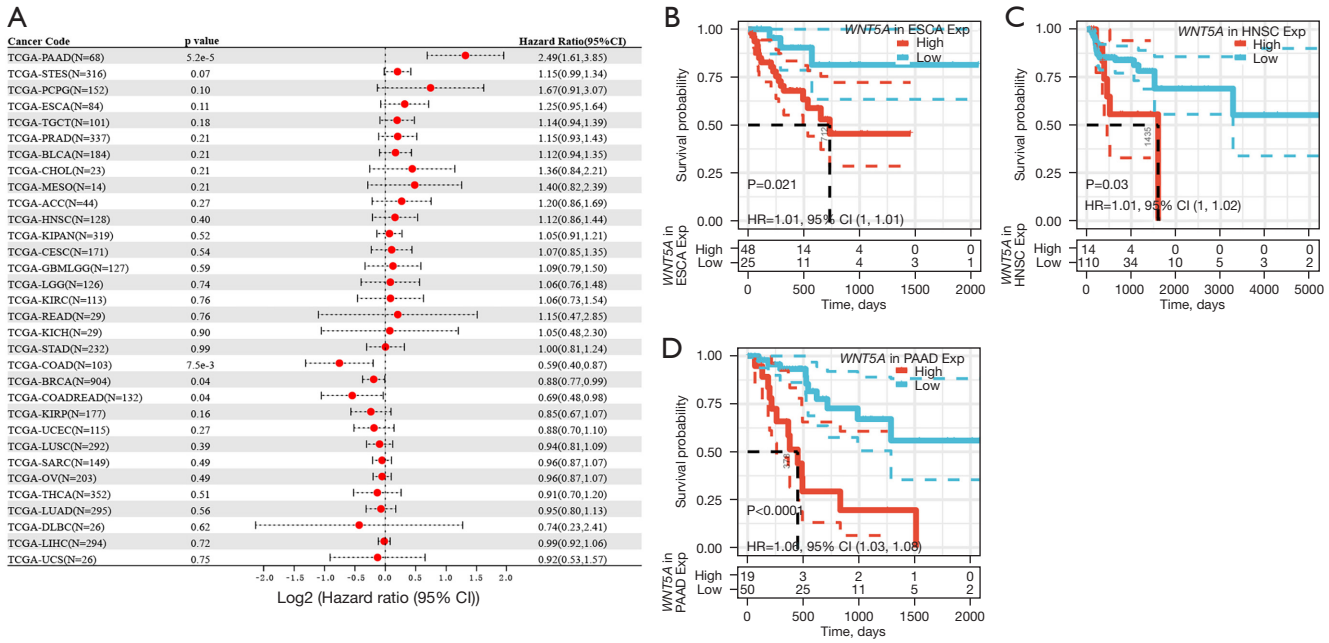

Figure 4 Associations between *WNT5A* expression and DFI. (A) Cox analysis of *WNT5A* expression with DFI in pan-cancer. (B-D) K-M analysis of *WNT5A* expression and DFI in ESCA, HNSC, and PAAD. DFI, disease-free interval; K-M, Kaplan-Meier; ESCA, esophageal carcinoma; HNSC, head and neck squamous cell carcinoma; PAAD, pancreatic adenocarcinoma.

were found simultaneously. Furthermore, among the data of immune cells, *WNT5A* expression was found to be positively associated with neutrophils and macrophages in 26 tumors, and DCs in 22 tumors (*Figure 6A*). In order to explore the effect of *WNT5A* expression on tumor microenvironment, we used the ESTIMATE algorithm to evaluate the correlation between *WNT5A* expression and stromal score. Results revealed the *WNT5A* expression was positively correlated with the stromal score in LUAD, GBMLGG, BRCA, COAD, KIRC, and PAAD (*Figure 6B*). In conclusion, these results demonstrate that *WNT5A* may promote immune cell infiltration in the tumor microenvironment (TME).

WNT5A is correlated with immune checkpoints and immune neoantigens in pan-cancer

The data presented above highlight a potential role for *WNT5A* in tumor immunity. Based on these findings, we performed correlation analysis of *WNT5A* expression and immune checkpoints, which included 24 immune inhibitors and 36 stimulators. Among the data of immune inhibitors in the 40 tumors, we found that *WNT5A* expression was positively linked to VEGFA in 23 tumors; to CD274 (PD-L1) in 20 tumors; to IL10 in 29 tumors; to CD276 in 34 tumors; to EDNRB in 29 tumors; to CTLA4 in 21 tumors; to IL12A in 22 tumors; to VTCN1 in 25 tumors; to TGFB1 in 28 tumors; to HAVCR2 in 26 tumors; to C10orf54 in 27 tumors; and to BTLA in 23 tumors. Additionally, among the data of immune stimulators, *WNT5A* expression was found to be positively associated with CX3CL1 in 23 tumors; HMGB1 in 28 tumors; ENTPD1 in 30 tumors; TLR4 in 32 tumors; tumor necrosis factor (TNF) SF4 in 33 tumors; BTN3A in 29 tumors; BTN3A2 in 23 tumors; CD40 in 25 tumors; ICAM1 in 26 tumors; IL1A in 29 tumors; IL1B in 27 tumors; TNF in 25 tumors; TNFRSF9 in 24 tumors; CD80 in 27 tumors; IL2RA in 28 tumors; ITGB2 in 23 tumors; CD28 in 27 tumors; and CD40LG in 24 tumors. Moreover, *WNT5A* expression was positively associated with 19 of 24 immune inhibitors and 29 of 36 immune stimulators in COADERAD; 17 of 24 immune inhibitors and 33 of 36 immune stimulators in neuroblastoma (NB); 18 of 24 immune inhibitors and 32 of 36 immune stimulators in PAAD; 17 of 24 immune inhibitors and 30 of 36 immune stimulators in uveal melanoma (UVM); 18 of 24 immune inhibitors and 28 of 36 immune stimulators in OV; 21 of 24 immune inhibitors and 30 of 36 immune stimulators in PRAD; 17 of 24 immune inhibitors and 31 of 36 immune

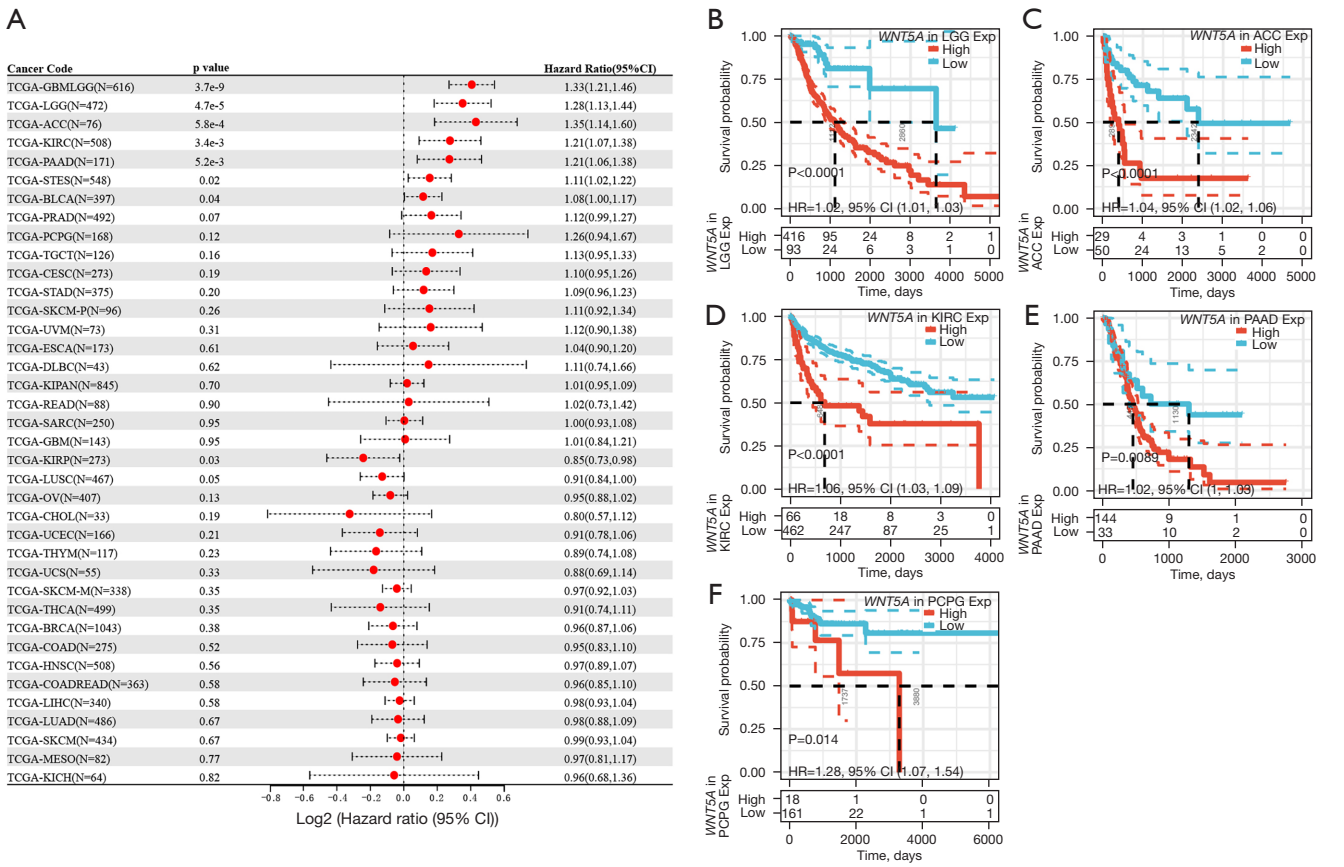

Figure 5 Associations between *WNT5A* expression and PFI. (A) Cox analysis of *WNT5A* expression with PFI in pan-cancer. (B-F) K-M analysis of *WNT5A* expression and PFI in LGG, ACC, KIRC, PAAD, and PCPG. PFI, progression-free interval; K-M, Kaplan-Meier; LGG, low-grade glioma; ACC, adrenocortical carcinoma; PAAD, pancreatic adenocarcinoma; KIRC, kidney renal clear cell carcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma.

stimulators in GBMLGG; and 18 of 24 immune inhibitors and 29 of 36 immune stimulators in LGG. Conversely, *WNT5A* expression was negatively associated with 10 of 24 immune inhibitors and 19 of 36 immune stimulators in LUSC, and 11 of 24 immune inhibitors and 17 of 36 immune stimulators in TGCT (*Figure 7A*). Next, results of neoantigens analysis suggested that *WNT5A* expression was negatively associated with the number of neoantigens in LUAD, LUSC, BRCA, UCEC, and SKCM, while positively associated with KIRP and HNSC (*Figure 7B*).

WNT5A is associated with TMB and MSI

Tumors are diseases caused by genetic mutations, while TMB and MSI can reflect the change of genomic instability (27). We found that *WNT5A* expression was positively correlated to TMB in ACC and OV, but negatively correlated to it in LUSC, ESCA, and READ (*Figure 8A*). Similarly, *WNT5A* expression was found to be positively associated with MSI in TGCT and ACC, but negatively associated with it in UCS, lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), and HNSC (*Figure 8B*).

WNT5A is implicated in the regulation of numerous signaling pathways

In order to clarify the relevant mechanisms, we firstly performed protein-protein interaction (PPI) network analysis to reveal the functional network of *WNT5A*. The results showed that *WNT5A* was linked to FZD2, FZD4, FZD5, FZD7, LRP5, LRP6, ROR2, RORA, DVL2, and RYK, most of which have been demonstrated to be related to the *WNT* signaling pathway (*Figure 9A*). Then GSEA was used to analyze the data of high and low

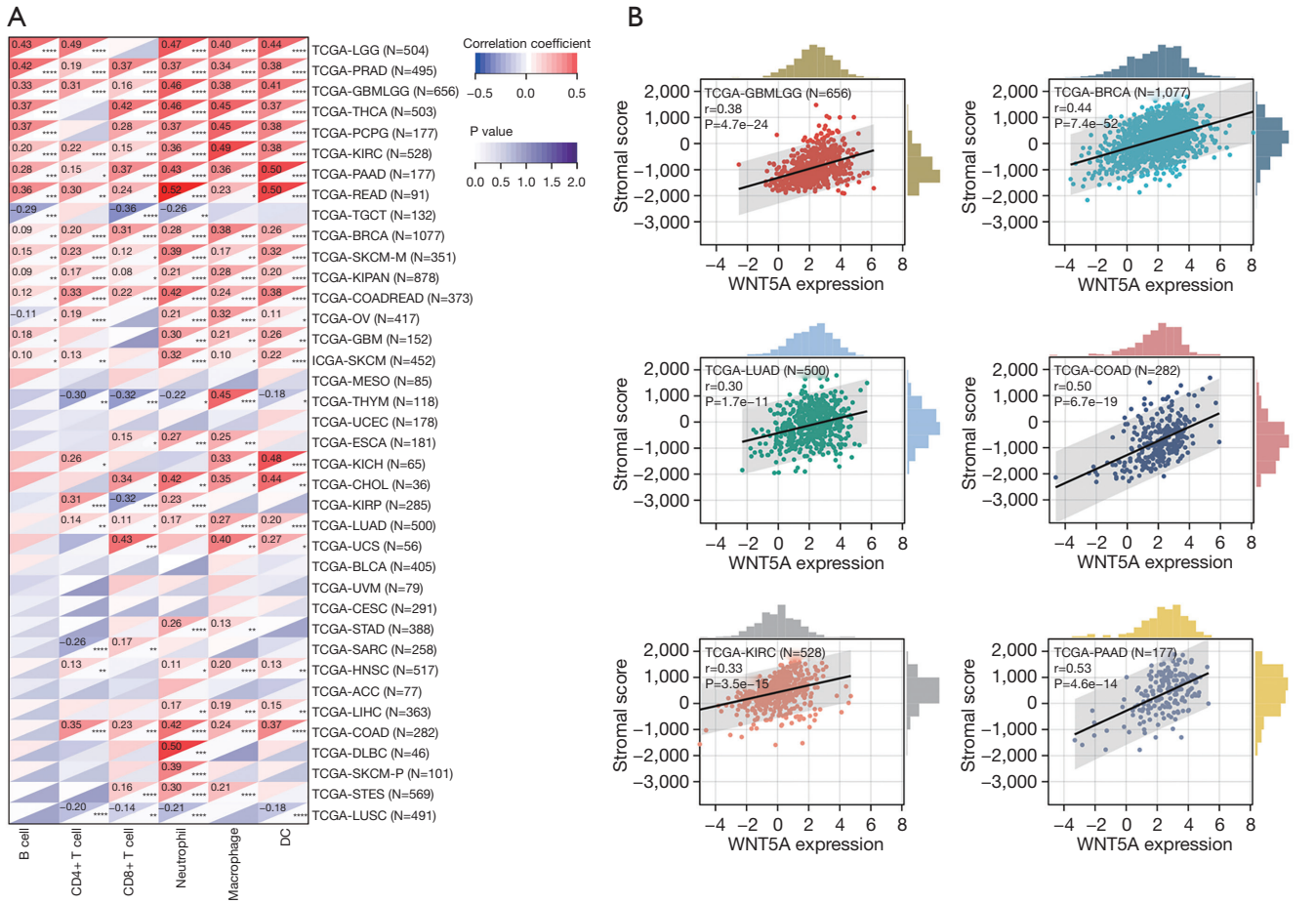

Figure 6 Correlations between *WNT5A* expression and tumor immune infiltration and TME. (A) Correlation analysis of the association between *WNT5A* expression and B cells, CD4$^+$ T cells, CD8$^+$ T cells, neutrophils, macrophages, and DCs in pan-cancer. (B) Relationship between *WNT5A* expression and stromal score. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. TME, tumor microenvironment; DCs, dendritic cells.

expression groups of *WNT5A*. The results indicated that the KEGG *WNT* signaling pathway (*Figure 9B*), KEGG basal cell carcinoma (*Figure 9C*), KEGG TGFβ signaling pathway (*Figure 9D*), hallmark epithelial-mesenchymal transition (EMT; *Figure 9E*), hallmark Hedgehog signaling (*Figure 9F*), and hallmark Notch signaling (*Figure 9G*) was highly enriched in the *WNT5A* high expression group.

Discussion

With the widespread use of immunotherapy and targeted therapy, the prognosis of tumor patients has improved (1). However, due to the heterogeneity of various patients, the OS of cancer patients remains poor (1,28). For this reason, the search for new therapeutic targets related to immunotherapy has received increasing attention from researchers. From another aspect, a pan-cancer analysis can provide broad insights about the role of a gene from many aspects in various cancers through mining major databases, which is an effective method to search for intriguing targets for tumor therapy (3).

As a non-classical *WNT* signal molecule, *WNT5A* is highly conserved between species and plays a key role in embryonic development, pathological disorders, and internal environmental balance (29). Due to its important role in embryonic development, the expression level of *WNT5A* is high in various organs and tissues during the embryonic stage, but generally decreased in adult tissues (7,30,31). It has been demonstrated that *WNT5A* expression increases when immune cells are exposed to pathogens (32).

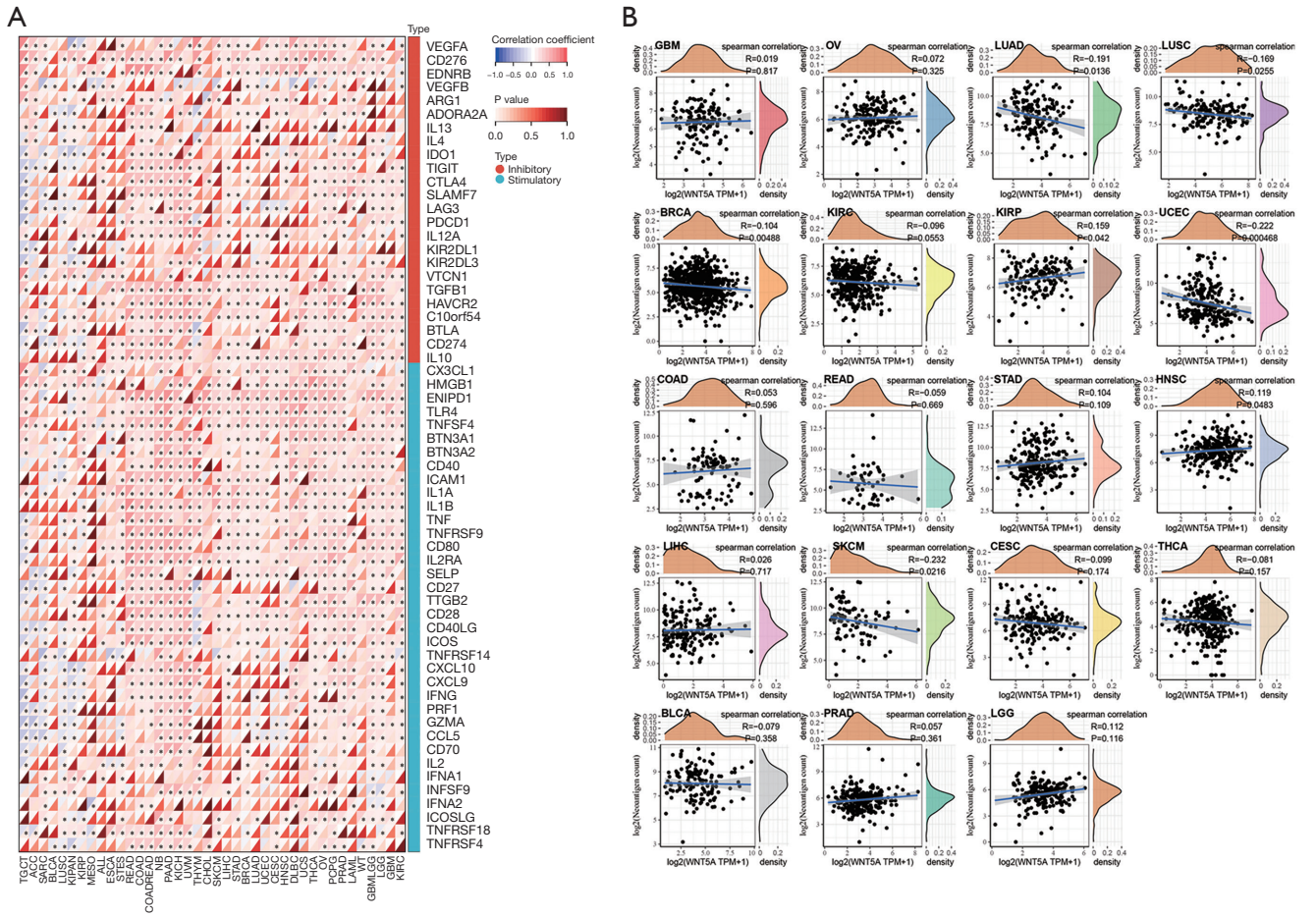

Figure 7 Correlations between *WNT5A* expression and immune checkpoints and tumor neoantigens. (A) Correlation analysis of the association between *WNT5A* expression and immune checkpoints (inhibitors and stimulators) in pan-cancer. (B) Correlation analysis of the association between *WNT5A* expression and the number of tumor neoantigens. *P<0.05.

Interestingly, our data from the GTEx dataset showed *WNT5A* was more highly expressed in the bladder, uterus, and vagina. As we know, these cavities, which are often exposed to bacteria, are prone to various forms of inflammation and immune cell congregation. Therefore, we can speculate that the high expression of *WNT5A* may be related to inflammatory cell infiltration and immune cells aggregation.

As a potential prognostic marker of cancer, *WNT5A* has anticancer or oncogenic activity, depending on tumor type and stages, and can regulate TME, inflammation, proliferation, EMT, and metabolism in cancer (7,33). Our analysis of the TCGA and GTEx datasets in 34 common tumors revealed that *WNT5A* expression was elevated in 19 tumors and lowered in 10 tumors, suggesting that it is overexpressed in most tumors but low expressed in some tumors. Among the positive results, the overexpression of *WNT5A* in some tumor species has been reported, such as LUSC (8), LUAD (8), ESCA (16), GBM (34), BRCA (35), PAAD (12), and ACC (36), while some of them have not been reported, such as GBMLGG, LGG, HNSC, and so on. Furthermore, combining previous literature with our survival analysis of OS, DSS, DFI, and PFI, among the *WNT5A* overexpression tumors, we found that *WNT5A* expression was associated with poor prognosis in ESCA (16), LGG, ACC (36), PAAD (12), and HNSC. Conversely, high *WNT5A* expression was correlated to longer DSS in KIRP, which warrants further research.

By analyzing recent studies on *WNT5A* and tumor immunity, Lopez-Bergami and Barbero proposed that *WNT5A* overproduced by the tumor cell could foster a pro-inflammatory milieu and induce immune cells chemotaxis.

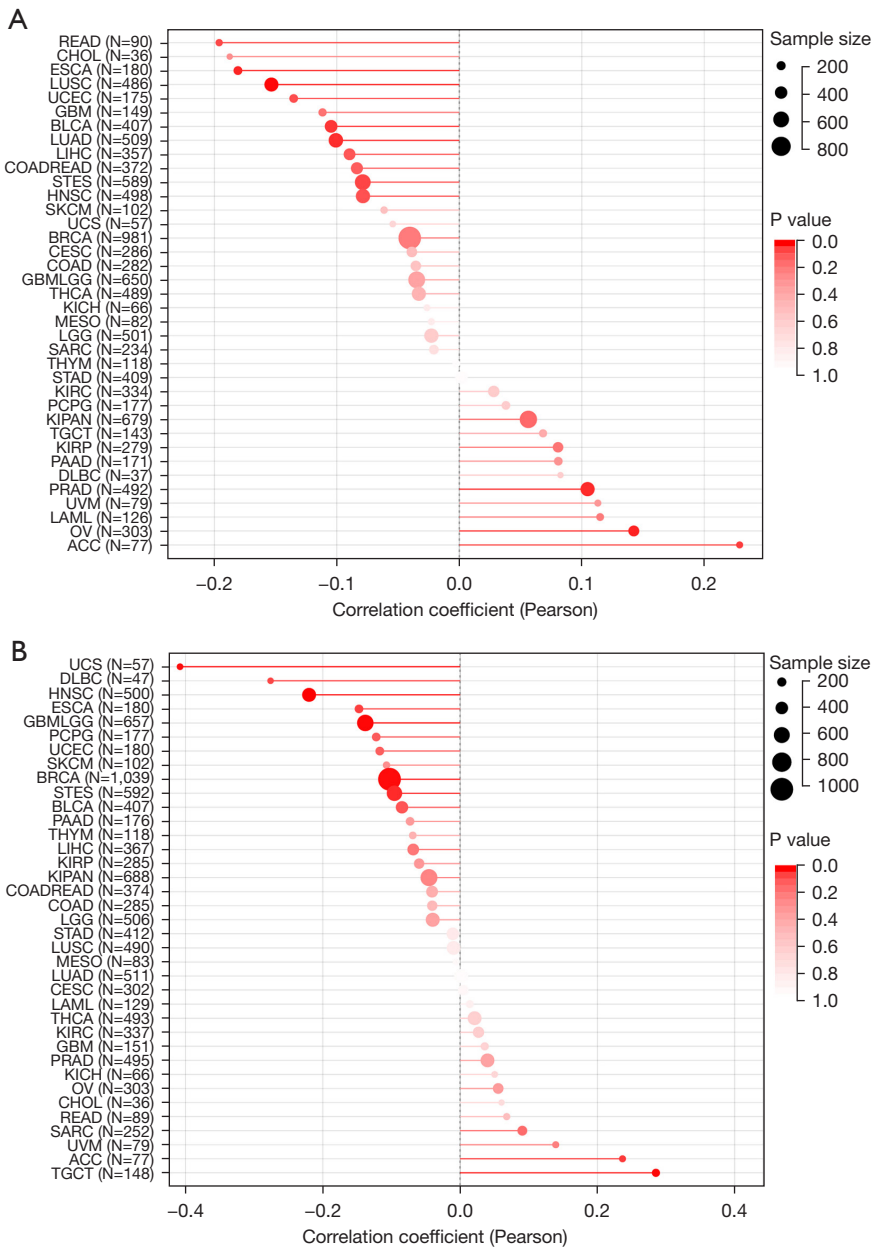

Figure 8 Correlations between *WNT5A* expression and TMB and MSI. (A) Correlation analysis of the association between *WNT5A* expression and TMB. (B) Correlation analysis of the association between *WNT5A* expression and MSI. TMB, tumor mutational burden; MSI, microsatellite instability.

When immune cells were recruited, *WNT5A* induced a tolerogenic phenotype of mononuclear phagocytes in myelomonocytic cells via the TLR/MyD88/P50 pathway (19,37). Our correlation analysis revealed that in most cancers, *WNT5A* expression was positively correlated with various immune cells, especially neutrophils, macrophages, and DCs. Otherwise, the correlation analysis using ESTIMATE algorithm showed that *WNT5A* expression was positively correlated with the stromal score in LUAD, GBMLGG, BRCA, COAD, KIRC, and PAAD. Those results showed that *WNT5A* expression may be associated with promoting inflammation, but as neutrophils, macrophages, and DCs also play an important role in immune suppression (23,38); the role of *WNT5A* in

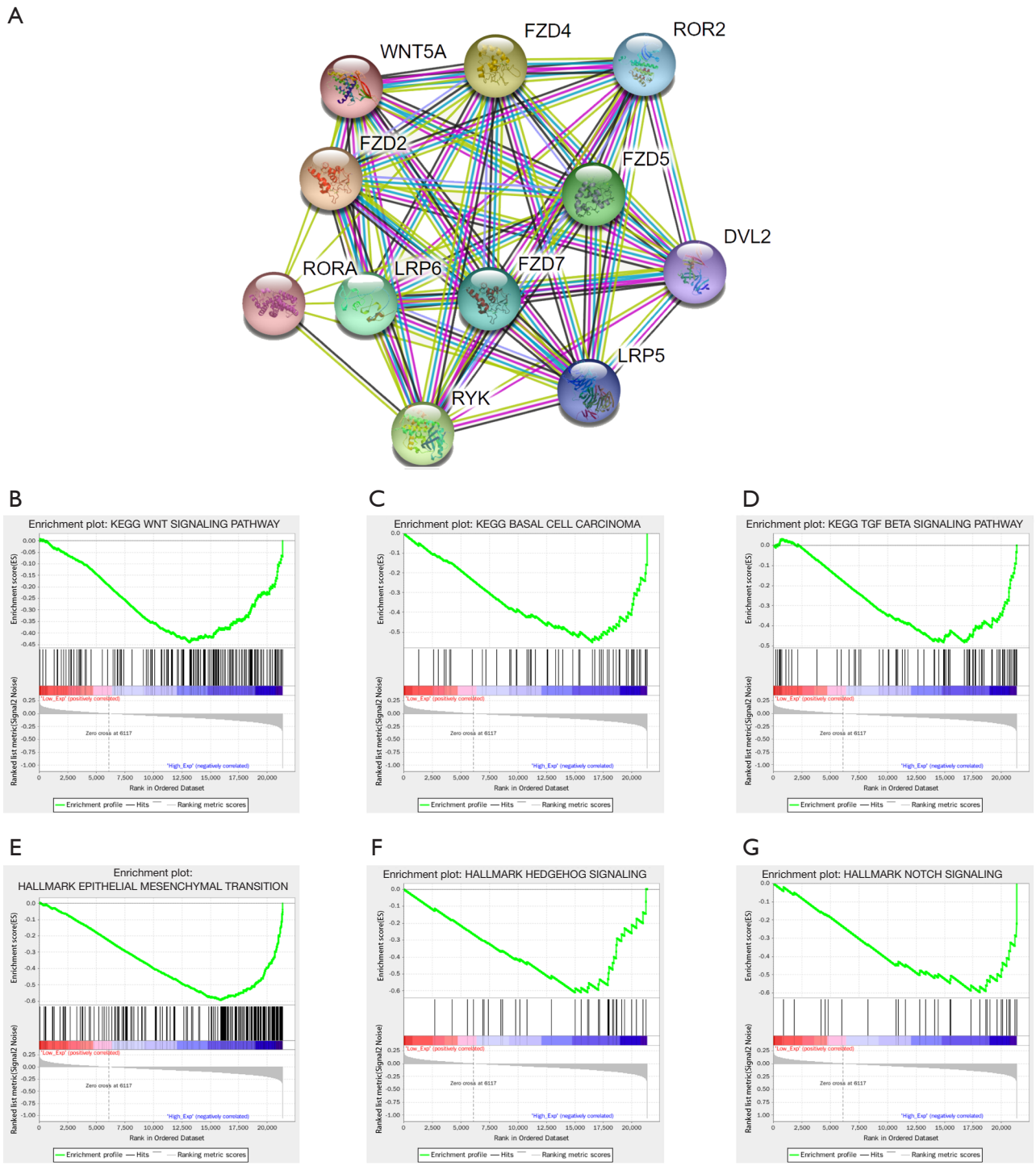

Figure 9 Signaling enrichment of *WNT5A* in KEGG and hallmark gene sets. (A) PPI network analysis of *WNT5A*. (B-D) GSEA analysis of the correlations between *WNT5A* and signaling pathways based on KEGG database. (E-G) GSEA analysis of the correlations between *WNT5A* and signaling pathways based on hallmark gene set. KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction; GSEA, gene set enrichment analysis.

promoting immune tolerance also needs to be noted.

To date, many immune checkpoints have been identified and studied, and *WNT5A* expression is thought to stimulate a variety of cytokines, including immune stimulators and inhibitors, which in turn cause inflammation or further stimulate immune tolerance (19,39). Our data revealed that *WNT5A* expression was positively linked to multiple immune inhibitors, such as VEGFA, PD-L1 (CD274), IL10, CD276, EDNRB, CTLA4, IL12A, and TGFB1, and various immune stimulators, such as HMGB1, ENTPD1, TLR4, TNFSF4, BTN3A, ICAM1, IL1A, IL1B, and TNF. Some of these cytokines have been reported to be associated with *WNT5A*, such as VEGFA (8), PD-L1 (22,37), IL10 (40), CTLA4 (23), IL1A (41), IL1B (41), and TNF (42). Furthermore, the results of neoantigen analysis suggested that *WNT5A* expression was associated with the number of neoantigens in LUAD, LUSC, BRCA, UCEC, SKCM, KIRP, and HNSC. Currently, PDL1 and CTLA4 are the main therapeutic targets of immunotherapy. Interestingly, *WNT5A* has been found to be associated with them, and inhibition of *WNT5A* can promote the effect of immunotherapy drugs (22,23), indicating that *WNT5A* may be a potential target of immunotherapy. In addition, we also found that the relationship between *WNT5A* expression and cytokines is not consistent in different tumors, suggesting the influence of tumor heterogeneity on *WNT5A*-targeted immunotherapy.

Both TMB and MSI tend to be predictive markers of immune checkpoint inhibitors (ICIs), which is important for identifying patients with potential for ICIs in various cancers (24,43). Our results revealed that *WNT5A* expression was positively correlated with TMB in ACC and OV, while negatively correlated to LUSC, ESCA, and READ. In addition, *WNT5A* expression was positively associated with MSI in TGCT and ACC, while negatively associated with MSI in UCS, DLBC, and HNSC. It is especially noteworthy that *WNT5A* expression was positively correlated with TMB and MSI in ACC. At present, the direct relationship between *WNT5A* and ACC has not been reported, but the carcinogenic effect of *WNT*/β-catenin in ACC has been revealed (44,45). Therefore, the relationship between *WNT5A* and tumor immunity in ACC warrants further confirmation.

The *WNT5A* gene could stimulate non-canonical *WNT* pathway as well as activate or antagonize the canonical *WNT* signaling pathway by binding to different receptors or co-receptor complexes, such as Frizzled (FZD), receptor tyrosine kinase-like orphan receptor-1 and 2 (ROR1/2), receptor related to tyrosine kinases (RYK), low-density lipoprotein receptor-related protein 5/6 (LRP5/6), and DVL, thus playing a crucial role in tumor development (29,46-48). Our PPI network analysis showed that *WNT5A* was linked to FZD2, FZD4, FZD5, FZD7, LRP5, LRP6, ROR2, DVL2, RYK, and RORA, most of which have been reported to be members of the *WNT* signaling pathway. Interestingly, there have been no direct studies on RORA and *WNT5A* until now, but it has been reported that RORA can encode the transcription activator RORα and further attenuates *WNT*/β-Catenin signaling in colon cancer (49,50), indicating the potential correlation between them, which needs to be further evaluated.

As an important molecule of the *WNT* signaling pathway, *WNT5A* can interact with TGFβ, Notch, or other pathways to regulate EMT and immunity in cancer (7,51,52). Our GSEA analysis also indicated that the KEGG *WNT* signaling pathway, KEGG basal cell carcinoma, KEGG TGFβ signaling pathway, hallmark epithelial-mesenchymal transition, hallmark Notch signaling, and hallmark Hedgehog signaling was highly enriched in the *WNT5A* high expression group. Previous studies have suggested the following functions: (I) *WNT5A* can regulate TGFβ1 to promote immunosuppression in melanoma (53); (II) in psoriasis, *WNT5A* and Notch1 signaling can influence each other and regulate the secretion of cytokines IL-12, IL-23, and TNF-α, which is related to immunity (54); (III) until now, no experimental reports on the interaction between *WNT5A* and Hedgehog signaling have been retrieved in the field of tumor immunity. However, it has been shown that Hedgehog signaling can mediate how *WNT*/β-catenin induces cartilage and bone tumor formation (55). Therefore, as a member of *WNT* family, the interaction between *WNT5A* and Hedgehog signaling in tumor immunity may be a feasible research direction.

In summary, we analyzed the expression and prognosis of *WNT5A* in different tumors, indicating that *WNT5A* is correlated with the prognosis of tumors. On this basis, we further revealed that *WNT5A* was associated with tumor immune, suggesting that it may be a potential immunological biomarker and therapeutic target in cancer. Of course, these conclusions were obtained by bioinformatical analyses of open accessible databases, for which there is a lack of experimental verification, but they still provide some evidence and have a certain significance for further research.

Acknowledgments

Funding: Our research was supported by the National Natural Science Foundation of China (Nos. 82173252, 81871866), the Shaanxi Social Development Science and Technology Key Project (Nos. 2016SF-308; 2019SF-033), Natural Science Foundation of Shaanxi Province (No. 2022JQ-862), and the Project of Tangdu Hospital, The Fourth Military Medical University (No. 2018 Key Talents).

Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-1317/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-1317/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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(English Language Editor: J. Jones)

Cite this article as: Feng Y, Wang Y, Guo K, Feng J, Shao C, Pan M, Ding P, Liu H, Duan H, Lu D, Wang Z, Zhang Y, Zhang Y, Han J, Li X, Yan X. The value of *WNT5A* as prognostic and immunological biomarker in pan-cancer. Ann Transl Med 2022;10(8):466. doi: 10.21037/atm-22-1317

