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Original Research

WDR4 is a Potential Indicator of Clinical Diagnostics, Prognosis, and Immunotherapy in Hepatocellular Carcinoma (HCC)

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Abstract

Background: In recent years, immunotherapy has gained increasing prominence in the treatment of hepatocellular carcinoma (HCC). However, effective immune-related biomarkers for HCC remain limited. In this study, both transcriptomic data and clinical information on HCC were obtained from The Cancer Genome Atlas (TCGA) database. **Methods**: The TIMER and GEPIA databases were used to validate the association between WDR4 expression and immune infiltration. Additionally, clinical and pathological data from patients who underwent single-agent immunotherapy for HCC were collected from Hunan Provincial People's Hospital (The First Affiliated Hospital of Hunan Normal University). The relationship between WDR4 expression levels, clinical pathological data, and patient prognosis was assessed using the Kruskal–Wallis test and Kaplan–Meier survival curve analysis. Spearman's correlation analysis was utilized to confirm the relationship between WDR4, CD68, and PD-L1 in HCC tissue. **Results**: *WDR4* was significantly upregulated in HCC tissues compared to para-carcinoma tissues ($p < 0.001$) and exhibited strong diagnostic potential. *WDR4* expression showed significant associations with various immune cells, including macrophages ($p < 0.001$). Kaplan–Meier survival analysis revealed that patients with high *WDR4* expression had shorter postoperative progression-free survival in the context of immunotherapy. Data from 37 patients who underwent postoperative single-agent immunotherapy for HCC demonstrated a significant correlation between *WDR*4 expression levels and disease-free survival (DFS), with strong statistical significance (log-rank $p < 0.001$). **Conclusions**: *WDR4* shows elevated expression in HCC tissues and is associated with immune infiltration, establishing it as a prognostic biomarker in HCC. Furthermore, the positive correlation observed between *WDR4* and CD68, as well as PD-L1 (*CD274*), underscores its potential as a guiding factor in immunotherapeutic approaches for HCC.

Keywords: hepatocellular carcinoma; TCGA; the *WDR4* gene; clinical prognosis; immunotherapy

1. Introduction

Primary liver cancer ranks as the fifth most prevalent malignancy globally and second in male mortality rates [1], with hepatocellular carcinoma (HCC) representing its predominant pathological subtype. Surgical resection remains historically effective for early-stage HCC [2], yet post-curative resection five-year recurrence exceeds 60% [3]. For advanced HCC, significant benefits have been achieved with targeted immunotherapies [4–6]. The REFLECT study [7] and the RATIONALE-301 study [8], for instance, have demonstrated promising clinical outcomes with lenvatinib targeted therapy and tislelizumab immunotherapy in the treatment of HCC. Despite molecular targeting and immunotherapy emerging as first-line treatment modalities for advanced HCC, overall survival and drug response rates remain dismally low. The high metastatic potential of HCC and its poor overall treatment outcomes remain major hurdles [9]. Additionally, the development of drug resistance significantly compromised patient prognoses [10,11]. Currently, clinically utilized immune checkpoint blockade (ICB) drugs primarily encompass anti-CTLA-4 and anti-PD-1/PD-L1 antibodies [12,13]. However, unlike squamous cell lung cancer, PD-L1 lacks validation as an immunotherapy biomarker in HCC. Asian HCC patients exhibit ~15% objective response rates (ORR) to ICB monotherapy, constrained by an immunosuppressive microenvironment enabling immune evasion [12,14]. Consequently, HCC lacks validated diagnostic/prognostic biomarkers and immunotherapy assessment criteria [15], necessitating exploration of molecular targets governing HCC pathogenesis, prognosis, and immune infiltration.

The *WD* repeat domain 4 (*WDR4*) gene, located at human chromosome 21q22.3, encodes a WD-repeat protein family member primarily implicated in cell cycle regulation, signal transduction, apoptosis, and gene expression modulation [16,17]. Elevated *WDR4* expression occurs in multiple malignancies including hepatocellular carcinoma (HCC), lung cancer, and esophageal cancer [18–20]. Func-

tioning as a core *m7G* modification subunit, *WDR4* critically regulates transcription, mRNA splicing/translation, and immune microenvironment remodeling [21–24]. The *MYC*-targeted *WDR4* pathway induces *CCNB1* translation, promoting HCC proliferation, metastasis, and sorafenib resistance [25]. Moreover, *METTL1/WDR4*-mediated tRNA m7G modification confers lenvatinib resistance in HCC [26]. Despite these findings, research remains predominantly focused on *METTL1* with limited investigation of *WDR4* [27]. Here, HCC datasets from TCGA were analyzed to elucidate *WDR4*'s significance. Utilizing R software (https://cran.r-project.org/bin/windows/Rtools/) and online databases, we assessed correlations between *WDR4* expression and clinicopathological features alongside immune cell infiltration, validating differential *WDR4* expression in cancerous versus paracancerous tissues via immunohistochemistry alongside PD-L1 and CD68 correlations.

2. Materials and Methods

2.1 Data Collection

The TCGA data portal (https://portal.gdc.cancer.gov/) provided HCC RNA-seq data and clinical information focused on *WDR4* expression [28]. Using R software, we integrated and deduplicated *WDR4* mRNA expression with clinical data, yielding three hundred sixty-nine HCC samples and fifty adjacent tissue samples. Concurrently, clinical data were collected from thirty-seven patients at Hunan Provincial People's Hospital (The First Affiliated Hospital of Hunan Normal University) who underwent HCC resection between January 2019 and December 2022 and received postoperative anti-PD-1 monotherapy. Inclusion criteria stipulated: ① All cases were diagnosed with primary HCC based on pathological examination; All cases underwent curative resection for HCC, followed by postoperative monotherapy (PD-1) immunotherapy. ② No targeted therapy, chemotherapy, or radiotherapy was administered before or after surgery; ③ No significant underlying comorbidities, secondary primary tumors, or immune-related diseases were present; ④ The medical records were complete, and follow-up data were comprehensive. Exclusion criteria: ① Patients diagnosed with other types of liver cancer (e.g., intrahepatic cholangiocarcinoma or mixed types); ② Hepatic metastases; ③ Patients who received preoperative antitumor treatments such as radiotherapy, chemotherapy, or local interventions were excluded; ④ Patients who did not receive monotherapy with immunotherapy after surgery, for example, those who received combined immunotherapy and targeted therapy or other antitumor treatments, were also excluded; ⑤ Patients with underlying diseases that severely affected their health were excluded; ⑥ Patients with immune-related underlying diseases were also excluded.

2.2 Gene Expression Analysis, Clinical Characteristics, and Prognostic Analysis

Utilizing HCC *WDR4* mRNA and clinical data from TCGA, differential expression analysis was conducted via R's "Limma" package [29], where genes with $|\log FC| > 1$ under the null hypothesis were defined as differentially expressed genes (DEGs): $\log FC < -1$ indicated downregulation in HCC versus adjacent tissues, while $\log FC > 1$ indicated upregulation. Immunohistochemistry (IHC) validated DEGs. The pROC package analyzed *WDR4* expression, with ggplot2 visualizing receiver operating characteristic (ROC) curves for patient assessment; the area under the curve (AUC) evaluated *WDR4's* diagnostic value for HCC, where values approaching 1 denote higher diagnostic efficacy. Xiantao Academic (https://www.xiantaozi.com) further analyzed associations between DEGs and clinicopathological characteristics including Stage, T stage, pathological grade, AFP levels, vascular invasion, and gender. Kaplan-Meier survival curves assessed relationships between *WDR4* expression levels and both overall and progression-free survival.

2.3 Analysis of the Relationship Between WDR4 Expression, Immune Infiltration, and Clinically Relevant Immune Markers

The TIMER2.0 database (http://timer.cistrome.org/) generated graphical representations of relationships between *WDR4* expression levels and various immune cells, cumulative survival rates, and immune-related cell interactions [30–32]. Kaplan-Meier survival curve analysis evaluated associations between immune cell infiltration levels and HCC patient prognosis. The GEPIA database [33] (http://gepia.cancer-pku.cn/) analyzed relationships between *WDR4* and immune cell molecular markers, including PD-1 (*PDCD1*: The gene name for PD-1), PD-L1, CTLA-4 and mismatch repair genes *MLH1*, *MSH2*, *MSH*, *PMS2*.

2.4 Gene Set Enrichment Analysis

The *WDR4* RNA sequencing data from HCC patients in the TCGA database were filtered and analyzed using R programming packages. The results were visualized and explored to investigate the various functions and signaling pathways associated with *WDR4* co-expressed genes in cancer [34]. $R > 0.3$ and $p < 0.05$ were considered statistically significant. The 20 selected *WDR4* co-expressed genes were subjected to analysis using the "clusterProfiler" package [35].

2.5 Immunohistochemistry Staining

Under informed consent, specimens from 37 postoperative HCC patients receiving single-agent immunotherapy were collected, including cancerous and adjacent tissues, along with clinical data. Immunohistochemical staining [25,36] was performed to assess the expression of *WDR4*, PD-L1, and CD68 in both cancerous and adjacent tissues.

The scoring criteria for *WDR4* and CD68 were based on staining intensity: a score of 1 ($\leq$25%), 2 (26%–50%), 3 (51%–75%), or 4 (76%–100%) was assigned according to the positive percentage of tumor-staining cells. Staining intensity was rated as 0 (no staining), 1 (weak staining), 2 (moderate staining), or 3 (strong staining). The staining index was calculated by multiplying the positive cell percentage and staining intensity, resulting in scores ranging from 0 to 12. Scores $\leq$6 were considered low expression, while scores >6 were deemed high expression. PD-L1 scoring was performed based on the proportion of positive tumor cells. Typically, 0–1% was considered a negative expression, and >1% was classified as a positive expression (For *WDR4* and CD68, positive controls were bile duct cancer tissues; for PD-L1, positive controls were tonsil tissues). Staining samples were independently and blindly reviewed by two experienced pathologists.

2.6 Statistical Analysis

The clinical data were statistically analyzed using R software version 4.2, GraphPad Prism 8.0 (https://www.ddooo.com/softdown/157257.htm), and Xiantao Academic (https://www.xiantaozi.com). The Wilcoxon rank-sum test was employed to assess the difference in *WDR4* expression between the two sample groups. An AUC value greater than 0.70 was considered indicative of significant diagnostic value. The Log-rank test was employed to validate the prognostic differences associated with *WDR4* in HCC patients, where $|r| \geq 0.3$ was considered indicative of a correlation. A significance level of $p < 0.05$ was considered statistically significant, and $p < 0.01$ was considered highly statistically significant.

3. Results

3.1 WDR4 Exhibits Elevated Expression Levels in HCC Tissues With Excellent Diagnostic Value

In this study, TIMER2.0 database analysis (Fig. 1A) revealed significant *WDR4* upregulation in multiple cancers, including bladder, breast, cervical squamous cell carcinoma, cholangiocarcinoma, colon, esophageal, head and neck squamous cell carcinoma, NK/T-cell lymphoma, HCC, lung adenocarcinoma, lung squamous cell carcinoma, pheochromocytoma, prostate, rectal adenocarcinoma, gastric adenocarcinoma, and endometrial carcinoma, with downregulation observed only in thyroid carcinoma. Further validation using TCGA data from 369 HCC and 50 para-carcinoma tissues demonstrated significant *WDR4* overexpression in HCC versus para-carcinoma tissues ($p < 0.001$, Fig. 1B). ROC curve analysis yielded an AUC of 0.778 (95% CI 0.734–0.822, Fig. 1C), indicating substantial diagnostic value of *WDR4* for HCC.

Furthermore, immunohistochemical analysis confirmed the nuclear localization of *WDR4*, as illustrated in Fig. 1D. Notably, *WDR4* exhibited high expression in HCC tissues while demonstrating low expression in para-carcinoma tissues. The expression patterns of *WDR4* in tumors and adjacent non-cancerous tissues from 37 HCC patients are summarized in Table 1. In HCC tissues, 10 cases (27.03%) exhibited low expression, while 27 cases (72.97%) showed high expression. In para-carcinoma tissues, 1 case (2.70%) displayed high expression, and 36 cases (97.30%) exhibited low expression. These results were statistically significant ($\chi^2 = 38.84$, $p < 0.001$). Overall, *WDR4* was highly expressed in HCC tissues that demonstrated excellent diagnostic value for HCC.

3.2 The Relationship Between WDR4 Expression in HCC Tissues and the Clinical Pathological Data of Patients

HCC occurrence and progression involve regulation by multiple genes and diverse factors; to elucidate the relationship between *WDR4* and HCC development, Kruskal-Wallis testing analyzed correlations between *WDR4* expression levels and patient clinical-pathological data (Fig. 2), revealing significantly increased *WDR4* expression in stage III versus stage II and I patients (Fig. 2A), higher levels in T2 and T3 versus T1 stages (Fig. 2B), elevated expression in pathological grades G3 and G4 compared to G1 and G2 (Fig. 2C), increased *WDR4* in patients with vascular invasion versus without (Fig. 2D), and higher expression associated with AFP levels >400 ng/mL relative to $\leq$400 ng/mL (Fig. 2E), although no significant correlation was observed with gender (Fig. 2F).

Clinical-pathological data from 37 postoperative HCC patients treated with single-agent immunotherapy were analyzed to validate the relationship between *WDR4* and clinical-pathological features. The results, as shown in Table 2, indicated a significant correlation between higher *WDR4* expression levels and higher pathological grades ($p < 0.001$), demonstrating a clear statistical difference. However, possibly due to the small sample size, this study did not find a significant association between *WDR4* expression and patients' age ($p = 0.395$), gender ($p = 0.412$), stage classification ($p = 0.350$), T classification ($p = 0.407$), or vascular invasion ($p = 0.503$). Despite the relatively limited overall dataset and the potential for selection bias, based on the aforementioned data, we can conclude that *WDR4* plays a certain role in the occurrence and development of HCC.

3.3 The Results of GO Enrichment and KEGG Pathway Enrichment Analyses

Using R software, 490 genes co-expressed with *WDR4* were identified and analyzed through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment. Results revealed significant roles for *WDR4* in hormone metabolism, vitamin D metabolism, and organic acid transport, as well as involvement in apical plasma membrane, basement membrane, and synaptic membrane formation (Fig. 3A). Molecular function analysis indicated primary activities in channel functions, passive transmembrane transporter activities, and signal-

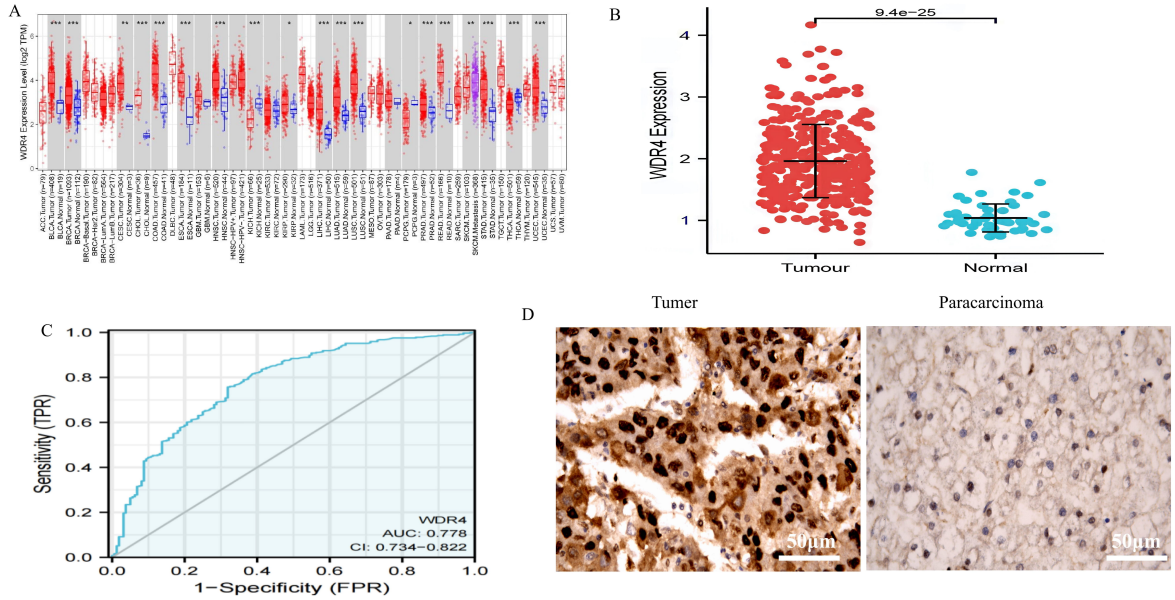

Fig. 1. The expression of WDR4 in various cancers in the TIMER2.0 and the expression and ROC curve analysis of *WDR4* in cancers including HCC. (A) The differential expression of *WDR4* in various tumor tissues and para-carcinoma tissues was analyzed using the TIMER2.0 database (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$). (B) The differential expression of *WDR4* between HCC tissues and para-carcinoma tissues was assessed using the Wilcoxon rank-sum test in the TCGA database. (C) ROC curve analysis was performed to assess the diagnostic value of *WDR4* in HCC. (D) Immunohistochemical staining revealed high expression of *WDR4* in liver cancer tissues and low expression in the matched para-carcinoma tissues (400×) (Scale bar: 50 μm). ROC, Receiver Operating Characteristic Curve; *WDR4*, *WD* repeat domain 4; HCC, hepatocellular carcinoma; TCGA, The Cancer Genome Atlas.

Table 1. The expression patterns of *WDR4* in HCC tissues and para-carcinoma tissues.

WDR4 expression level	HCC tissues		Para-carcinoma tissue		χ^2-value	*p*-value
	Number (n)	Percent (%)	Number (n)	Percent (%)		
Low	10	27.03%	36	97.30%	38.84	<0.001
High	27	72.97%	1	2.70%		

ing receptor activator activities. KEGG analysis demonstrated enrichment in neuroactive ligand-receptor interactions, retinol metabolism, cytochrome P450-mediated drug metabolism pathways, and chemical carcinogenesis-DNA adducts (Fig. 3B).

3.4 Correlation Between WDR4 Expression Level and Infiltration Degree of Immune Cells

To investigate the relationship between *WDR4* and immune infiltration, the TIMER database was used to analyze correlations between *WDR4* expression and immune cell infiltration in HCC (Fig. 4A). The results revealed a positive correlation between *WDR4* expression in HCC and the infiltration levels of $CD4^+$ T cells, B cells, macrophages, dendritic cells, lymphoid progenitor cells, and myeloid-derived suppressor cells ($p < 0.001$). But no significant correlation was found between *WDR4* expression and $CD8^+$ T cell infiltration levels ($p > 0.05$). These findings underscore the complex interplay between *WDR4* expression and immune cell infiltration within the tumor microenvironment.

In pursuit of a deeper understanding of the influence of immune cell infiltration on cumulative survival rates in HCC, this study delved into the relationship between immune-related cells and patients' overall survival using the TIMER database. Through meticulous analysis, we uncovered intricate connections between immune cell infiltrates and the cumulative survival outcomes of patients afflicted with HCC. As depicted in Fig. 4B, significant correlations between immune cell infiltration and the 120-month cumulative survival rates of HCC patients were revealed. The cumulative survival (120) was positively correlated with $CD8^+$ T cells ($p = 0.0408$) and the infiltration of macrophages ($p < 0.001$), but negatively correlated with myelosuppressive cells and tumor-associated fibroblasts ($p < 0.05$). Notably, no substantial correlations were observed between B cells, $CD4^+$ T cells, dendritic cells, myeloid progenitor cells, and lymphoid progenitor cells with regard to infiltration levels. Thus, we concluded that the *WDR4* expression level has a co-relationship with macrophages and myelmarrow pressive cells, thereby affecting the prognosis of HCC.

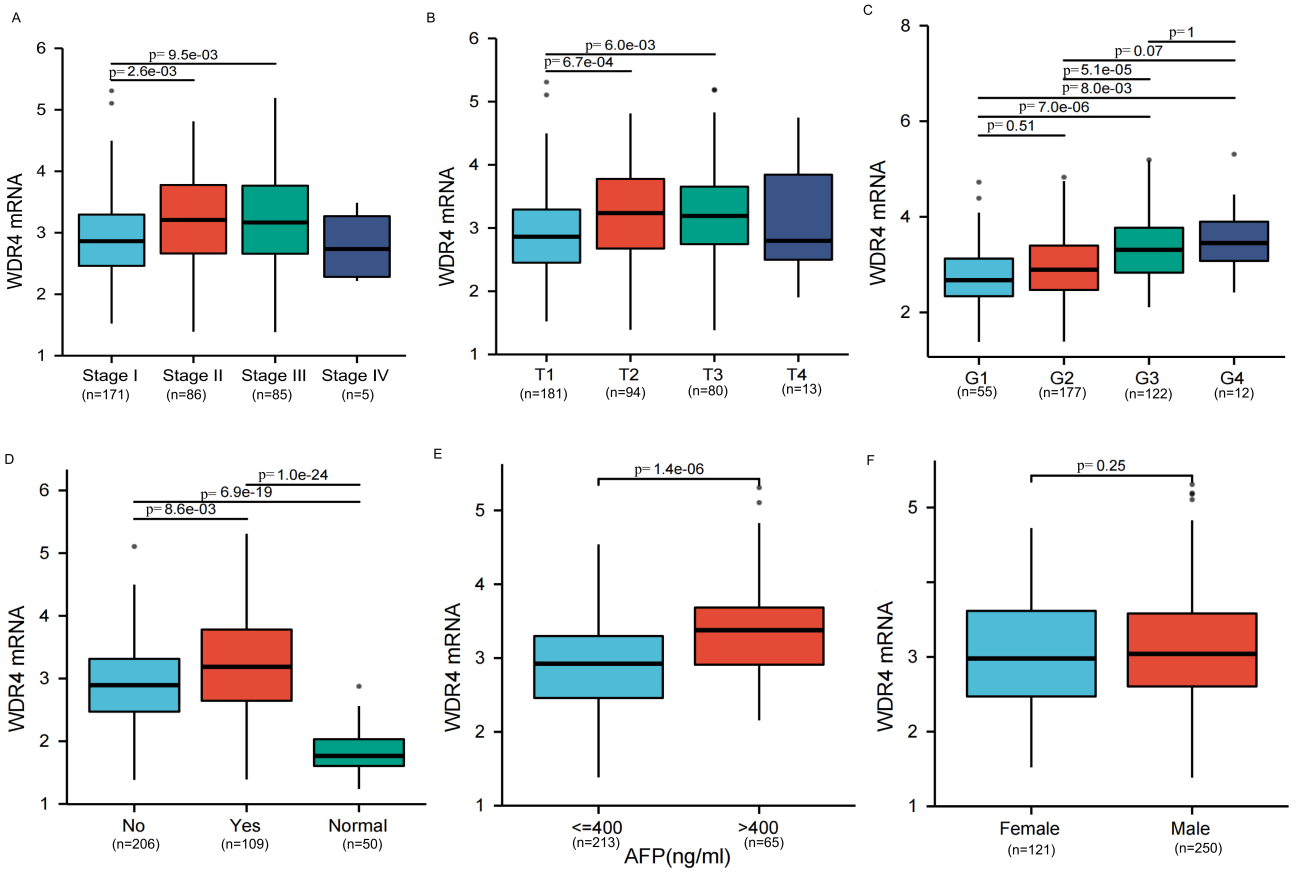

Fig. 2. The relationship between *WDR4* mRNA expression levels and the clinical stage, grade, and gender of HCC patients. (A) The relationship between *WDR4* mRNA expression levels and the clinical stage of HCC patients. (B) The association between *WDR4* mRNA expression levels and the T-stage classification of HCC patients. (C) The relationship between *WDR4* mRNA expression levels and the pathological grading of HCC patients. (D) The relationship between *WDR4* mRNA expression levels and vascular invasion in HCC patients (NO: representing no vascular invasion; YES: indicating presence of vascular invasion; Normal: para-carcinoma tissue). (E) The correlation between *WDR4* mRNA expression levels and AFP values in HCC patients. (F) The relationship between *WDR4* mRNA expression levels and gender in HCC patients.

We utilized the *GEPIA* database to investigate the interplay between *WDR4* and macrophages, as well as clinically relevant immune molecular markers. Comprehensive analysis of *WDR4* expression in HCC patients revealed associations with immune cell-related molecular markers. Table 3 shows significant correlations between *WDR4* expression in HCC tissues and specific macrophage-related markers. Notably, *CCL2* (a tumor-associated macrophage marker) exhibited significant relevance ($p = 0.012$), while IRF5 (an M1 macrophage marker) showed substantial positive correlation ($p < 0.001$). No significant correlations were observed with *NOS2* ($p = 0.290$) or *COX2* ($p = 0.210$). Significant positive correlations existed with M2 macrophage markers CD163 ($p < 0.001$) and MS4A4A ($p < 0.001$). Fig. 4C demonstrates associations between *WDR4* expression and immune-related markers including PD-1, PD-L1, CTLA-4, CD68, and mismatch repair genes *MLH1*, *MSH2*, *MSH6*, *PMS2* (all $p < 0.001$). These findings highlight significant positive correlations between *WDR4* expression and immune checkpoints and mismatch repair genes, illuminating its role in immune regulation in HCC.

Our findings demonstrate a significant correlation between *WDR4* expression and immune responses in HCC, particularly its substantial association with immune cells like macrophages and clinically relevant immune markers, underscoring *WDR4*'s pivotal role in HCC immune infiltration and immunological landscape.

3.5 Correlation Between WDR4 Expression and the Macrophage Marker CD68, as Well as PD-L1, in HCC Tissues

To delve deeper into the intricate interplay between *WDR4*, immune-related markers, and macrophages, we conducted a rigorous Spearman correlation analysis on 37 HCC tissue samples. Clinical and pathological data from patients who underwent single-agent immunotherapy for HCC were collected from Hunan Provincial People's Hos-

Table 2. The summary of the relationship between *WDR4* and clinical case data of HCC patients.

Clinical feature	N	*WDR4* expression level		*p*-value
		Low	High	
Gender				0.412
Male	32	8	24	
Female	5	2	3	
Age				0.395
≤55 year	18	4	14	
>55 year	19	6	13	
Pathological grade				<0.001
Medium-high differentiation	11	2	9	
Medium differentiation	22	8	14	
Low-medium differentiation	4	0	4	
Stage staging				0.350
I	14	6	8	
II	2	0	2	
III	17	3	14	
IV	4	1	3	
T stage				0.407
T1	15	6	9	
T2	2	0	2	
T3	8	1	7	
T4	12	3	9	
Vascular invasion				0.503
No	24	7	17	
Yes	13	3	10	

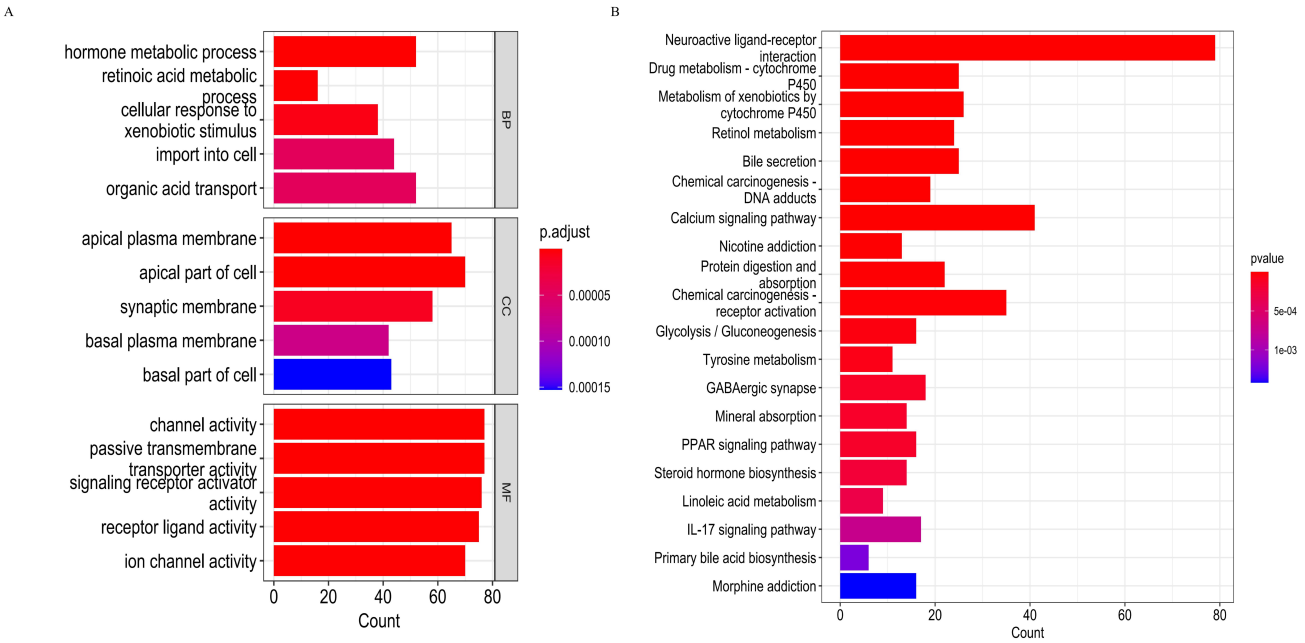

Fig. 3. GO and KEGG of *WDR4*. (A) GO functional enrichment analysis of *WDR4*. (B) KEGG functional enrichment analysis of *WDR4*.

pital (The First Affiliated Hospital of Hunan Normal University). The results unveiled a significant positive correlation between *WDR4* expression levels and key macrophage markers, including CD68 and PD-L1. As illustrated in Table 4 and Fig. 5, the expression levels of CD68 and PD-L1 exhibited a similar trend to that of *WDR4*, in both patients with high and low *WDR4* expression. These findings strongly suggest that *WDR4* may indeed be positively as-

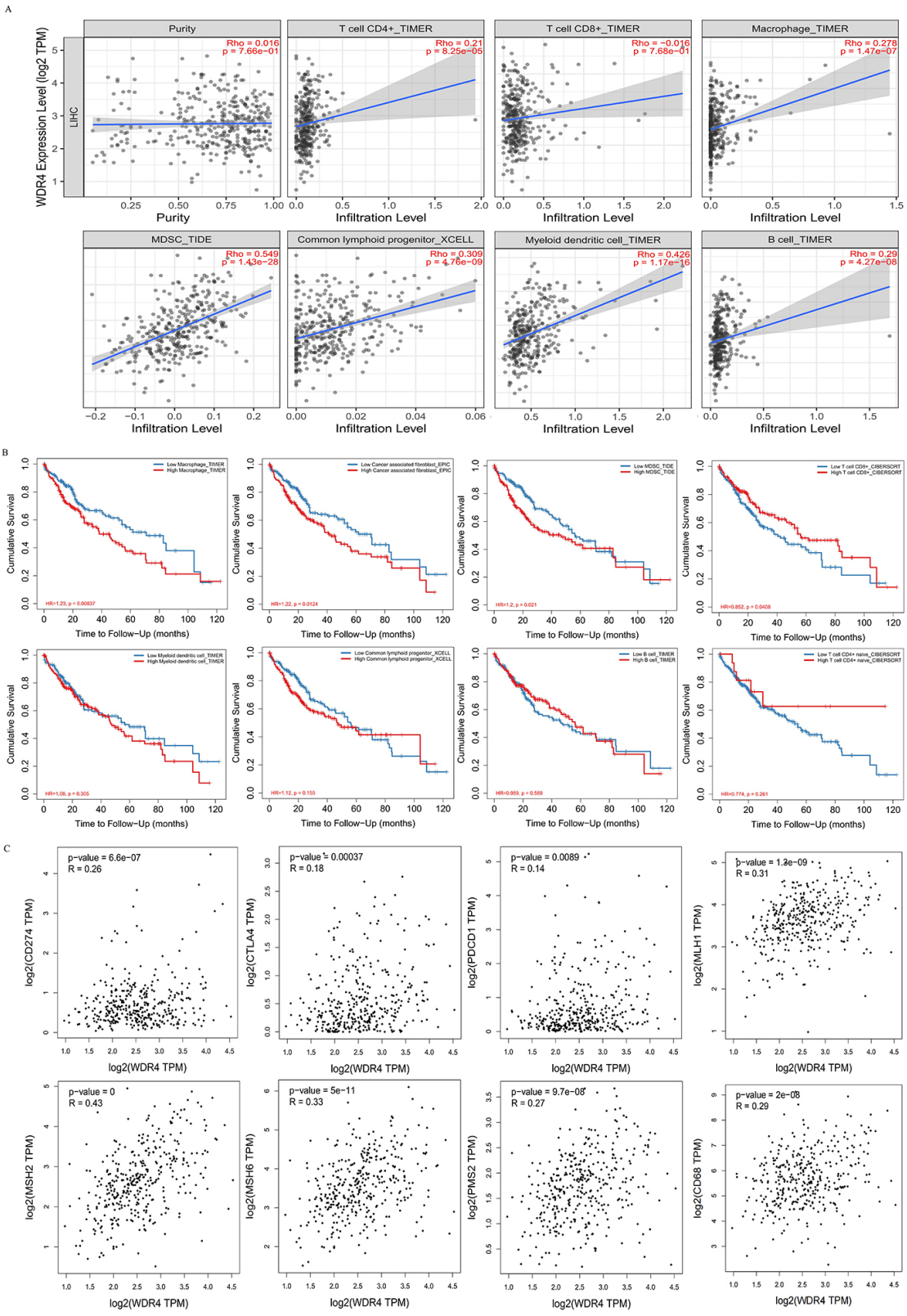

Fig. 4. Illustration of a compelling association between *WDR4* expression in HCC patients and various immune-related markers. (A) Plots showing the relationship between *WDR4* expression levels and $CD4^+$ T cells, $CD8^+$ T cells, macrophages, myeloid inhibitor cells, lymphoid progenitor cells, dendritic cells, and B cells. (B) The cumulative survival rate of HCC patients is correlated with the degree of infiltration of macrophages, tumor-associated fibroblasts, dendritic cells, $CD8^+$T cells, lymphoid progenitor cells, B cells, $CD4^+$ T cells, and myeloid progenitors. (C) Correlations between *WDR4* expression Levels in HCC and PD-L1, CTLA-4, PD-1, MLH1, MSH2, MSH6, PMS2, CD68 in the GEPIA Database.

Table 3. Correlations between *WDR4* expression levels in HCC and various immune cell markers in GEPIA database.

Cell type	Cell markers	Hepatocellular carcinoma			
		Tumour		Nontumorous	
		r	*p*	r	*p*
Tumor-associated macrophages	CCL2	0.130	0.012	0.340	0.015
M1 type macrophages	INOS(NOS2)	–0.055	0.290	0.032	0.820
	IRF5	0.250	<0.001	0.490	<0.001
	COX2(PTGS2)	0.066	0.210	0.290	0.042
M2 type macrophages	CD163	0.230	<0.001	0.550	<0.001
	MS4A4A	0.230	<0.001	0.480	<0.001

Table 4. Correlations between *WDR4* Expression and CD68 as well as PD-L1 in HCC tissues.

	Statistical results	*WDR4*
CD68	Spearman correlation analysis	0.528
	Significance (double-tailed)	<0.001
	N	37
PD-L1	Spearman correlation analysis	0.509
	Significance (double-tailed)	<0.001
	N	37

sociated with macrophages and PD-L1. This intricate relationship underscores the potential immunomodulatory role of *WDR4* in the context of HCC.

3.6 Elevated WDR4 Expression Correlates With Poor Clinical Prognosis in HCC Patients

To investigate the relationship between *WDR4* expression and HCC prognosis, we analyzed overall survival using R software, with Kaplan-Meier curves demonstrating significantly shorter survival in high-*WDR4*-expression patients versus low-expression counterparts (n = 371, p < 0.001, Fig. 6A); concurrently, progression-free survival was markedly prolonged in low-*WDR4*-expression patients (n = 371, p < 0.001, Fig. 6B). Through the GEPIA database that PD-L1 expression had no significant correlation with the DFS of patients with liver cancer (n = 181, p = 0.55, Fig. 6C).

In the final validation step, we analyzed data from 37 patients post-immunotherapy with PD-1 inhibitors, where Kaplan-Meier survival analysis of disease-free survival revealed a significant correlation between *WDR4* expression levels and patient outcomes after single-agent immunotherapy (Log-rank p < 0.001), consistent with earlier bioinformatics findings (Fig. 7A). Additionally, we investigated PD-L1 expression, which showed no significant DFS correlation (Log-rank p = 0.385, Fig. 7B). These results underscore the predictive power of *WDR4* expression for immunotherapeutic interventions, reaffirming its potential as a crucial biomarker for guiding HCC treatment strategies, while collectively, the consistent findings across diverse analyses highlight the robustness and reliability of *WDR4* as a prognostic indicator in HCC immunotherapies.

4. Discussion

For its highly malignant nature, insidious progression and frequent recurrence, hepatocellular carcinoma (HCC) demonstrates poor treatment outcomes, underscoring the critical importance of identifying effective diagnostic indicators and therapeutic biomarkers [37]. While alpha-fetoprotein (AFP) represents the most common serological HCC diagnostic marker [38], approximately 30–40% of patients lack AFP elevation. Conversely, nonmalignant conditions including chronic liver disease and reproductive system tumors can elevate AFP levels, limiting its diagnostic specificity [39]. Although studies link AFP to HCC immunotherapy response [40], immune tolerance and other factors preclude its utility as a reliable immunotherapy biomarker [38]. *WDR4*, a WD repeat protein family member, exhibits overexpression in hepatoblastoma, lung cancer, pancreatic cancer, and head/neck malignancies, implicating it in cancer initiation, progression, metastasis and adverse prognosis [18–20,41]. *WDR4* cooperates with *METTL1* to maintain m7G methyltransferase activity [42], with *METTL1/WDR4*-mediated m7G tRNA methylation proving pivotal for mouse embryonic stem cell self-renewal and differentiation [17]. *WDR4* potentially engages in protein-protein interactions and ligand binding, with triplication in trisomy 21 potentially contributing to Down syndrome phenotypes [16]. Additionally, defects in the *WDR4-ARHGAP17-Rac1* signaling pathway may associate with Spearson cerebellar developmental disorders [43]. Ma *et al.* [19] demonstrated that *WDR4/METTL1* knockdown impairs m7G tRNA modification, suppressing proliferation and invasion, whereas overexpression promotes tumorigenesis. This study reveals significantly elevated *WDR4* expression in HCC versus adjacent tissues, correlating with metastasis and poor prognosis, positioning *WDR4* as an oncogene with diagnostic and adverse prognostic biomarker potential in HCC.

Cancer development is recognized as an evolutionary ecological process [44]. The tumor microenvironment (TME), constituting the milieu for tumor growth, is piv-

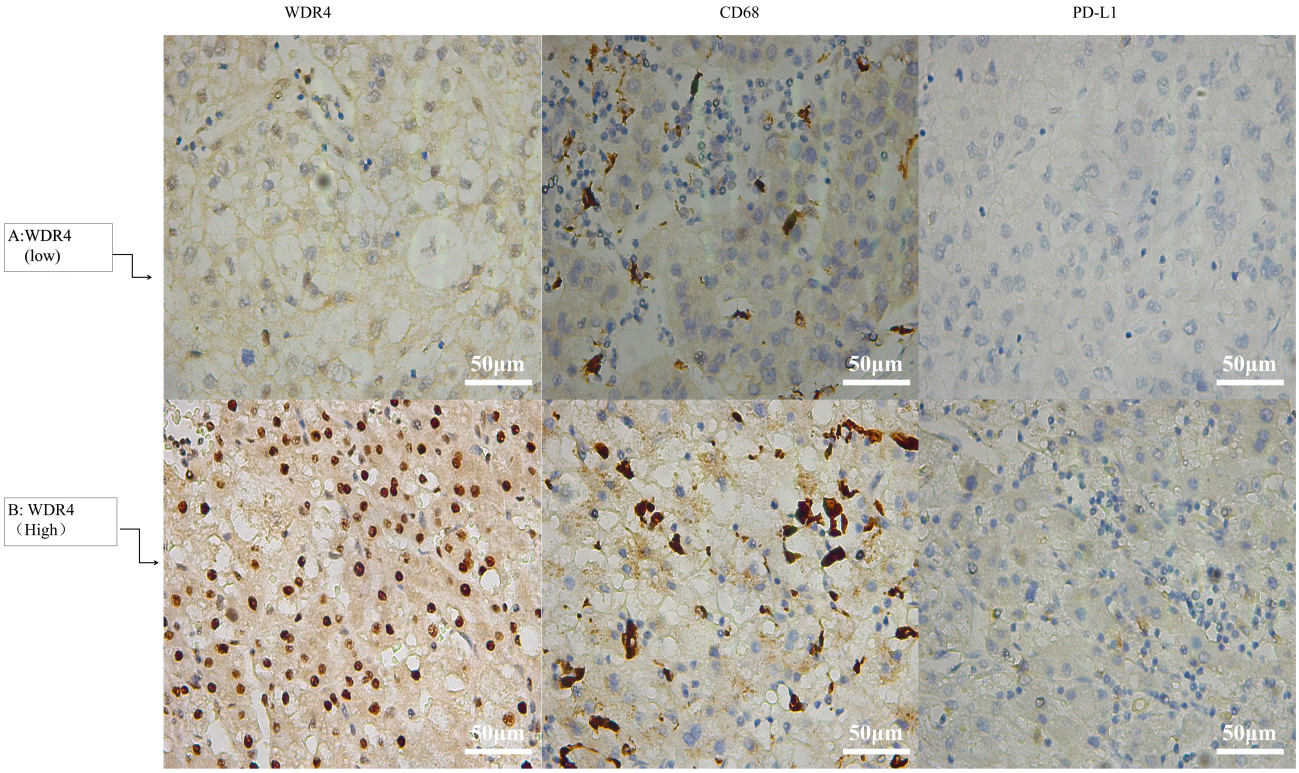

Fig. 5. Expression levels of *WDR4*, CD68, and PD-L1 in HCC tissues (400×). (A) From left to right, the expression of *WDR4*, CD68 and PD-L1 in patients with low *WDR4* expression. (B) From left to right, the expression plots of *WDR4*, CD68, and PD-L1 in patients with high *WDR4* expression. Scale bar: 50 μm.

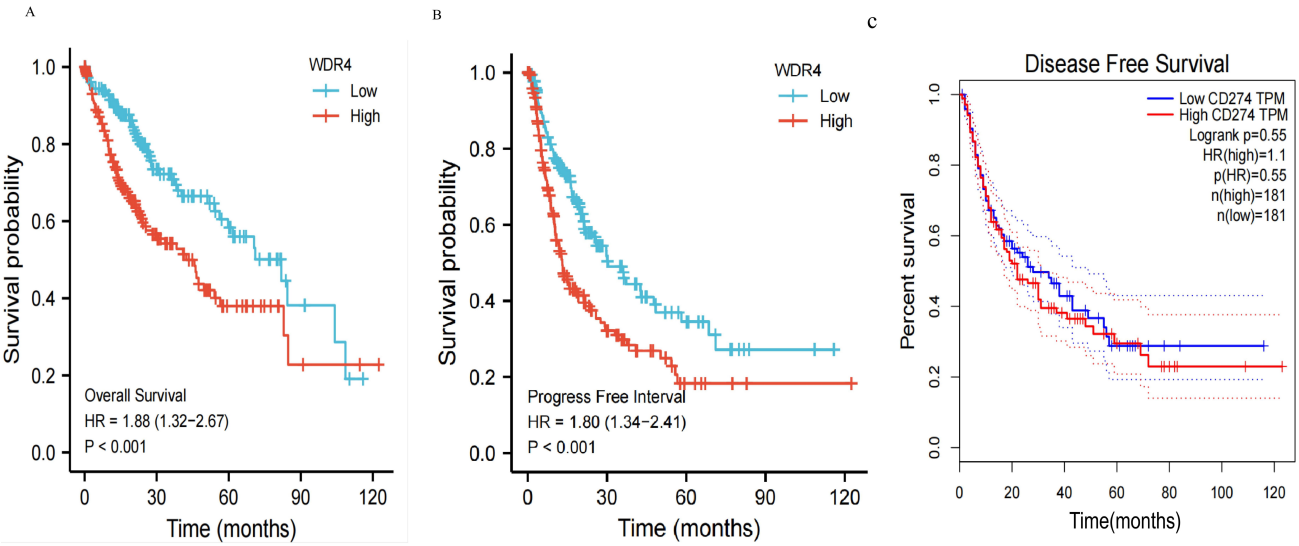

Fig. 6. Elevated *WDR4* expression correlates with poor OS and PFS clinical prognosis in HCC patients, but the expression of PD-L1 has no significant correlation with the DFS of HCC patients. (A) Kaplan-Meier survival curves for OS of *WDR4* high and low expression groups versus HCC patients in the TCGA database. (B) Kaplan-Meier survival curve for PFS of *WDR4* expression in HCC patients from TCGA database. (C) Kaplan-Meier survival curve for DFS of PD-L1 expression in HCC patients from GEPIA database.

otal in tumor initiation and progression. The TME comprises all non-cancerous host cells and non-cellular components, including fibroblasts and immune cells [45]. Recent studies [46,47] highlight crucial roles for macrophages and stromal fibroblasts in tumor initiation, with the TME intricately linked to tumor formation, sustenance, and metastasis. Research on the HCC immune microenvironment is advancing, with breakthroughs in targeted angiogenic

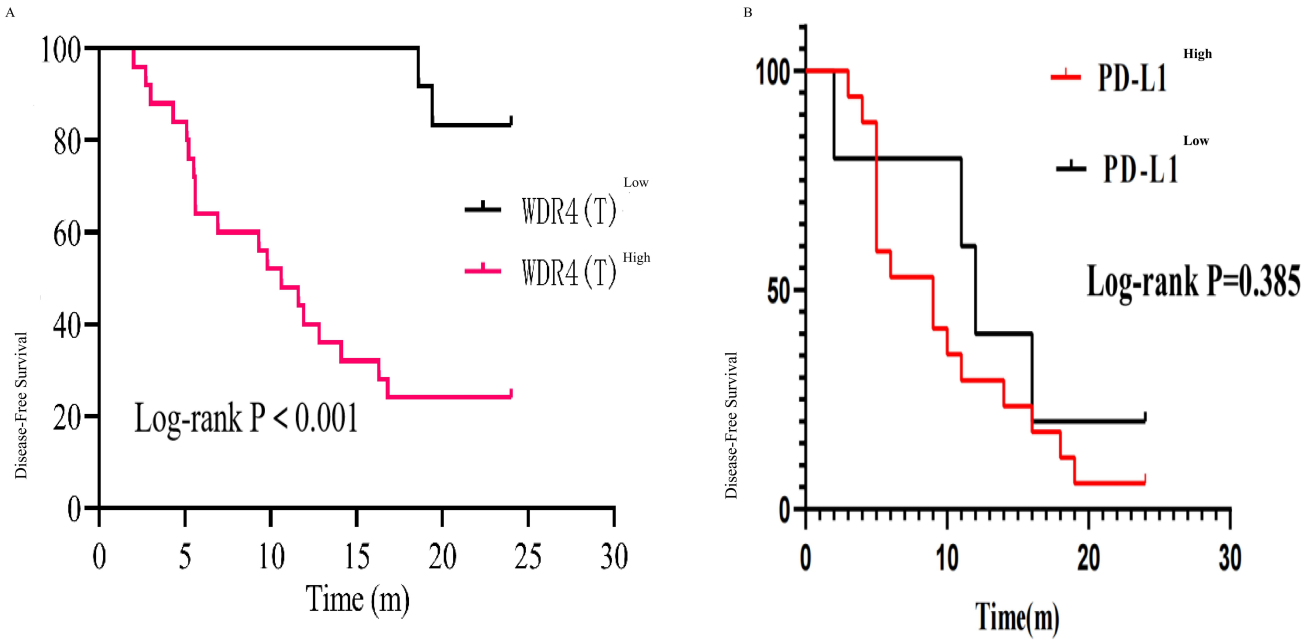

Fig. 7. Elevated *WDR4* or PD-L1 expression correlates with Disease-Free Survival (DFS) clinical prognosis in HCC patients. (A) Kaplan-Meier survival curves of high and low *WDR4* expression groups and DFS in HCC tissues. (B) Kaplan-Meier survival curves for high-and low PD-L1 expression groups and DFS in HCC tissues. Data from 37 HCC samples that underwent single-agent immunotherapy were collected from Hunan Provincial People's Hospital (The First Affiliated Hospital of Hunan Normal University).

drugs; combined targeted immunotherapy outperforms sorafenib [48]. Nevertheless, the HCC drug arsenal remains limited, and drug resistance compromises overall treatment efficacy. Immunoinfiltration plays a pivotal role in tumor initiation, progression, metastasis, and drug resistance [18–20,41,49]. *WDR4* contributes to renal cancer's immune microenvironment, where its knockout inhibits proliferation and enhances sunitinib/sorafenib sensitivity in 786-0 and Caki-1 cells [24]. Additionally, *WDR4* negatively regulates Promyelocytic Leukemia (PML) via ubiquitination, promoting immune suppression and shaping the TME to facilitate lung cancer progression [49]. Here, we observed positive correlations between *WDR4* expression in HCC and infiltration levels of macrophages, tumor-associated fibroblasts, and other immune cells. Further analysis linked immune cell infiltration to cumulative 120-month survival, revealing a negative correlation for macrophages. These findings associate *WDR4* with macrophage, tumor-associated fibroblast, and myeloid-derived suppressor cell infiltration in liver cancer, ultimately affecting prognosis.

Immunotherapy has become the fourth pillar of cancer treatment alongside surgery, chemotherapy and radiation [50]. Immune checkpoint inhibitors targeting PD-1, PD-L1, CTLA-4 and mismatch repair genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*) represent pivotal benchmarks in cancer immunotherapy. However, monotherapy immunotherapy shows inferior efficacy to targeted therapy in first-line HCC treatment [51]. Unlike other solid tumors, studies show no established correlation between tumor cell PD-L1 expression and anti-PD-1 inhibitor response in HCC [12], potentially due to HCC's complex immune microenvironment. The liver harbors approximately 80% of body macrophages that scan and infiltrate the vasculature [52]. Tumor-associated macrophages (TAMs), the most abundant immune infiltrates in the TME, critically influence HCC through phenotypic diversity [53]. As integral components of the immune microenvironment, macrophages contribute indispensably to innate and adaptive immunity [54]. Although typically tumoricidal, macrophages also exhibit tumor-promoting effects: $CD68^{+}$ MI macrophage-infiltrated hepatoma cells induce PD-L1 overexpression [55], and TAMs induce immunosuppression within the *HCC TME* [56]. Despite expanding research on HCC macrophages, effective immunotherapy biomarkers remain elusive. Recent pan-cancer analyses reveal aberrant *WDR4* expression correlating with immune cell infiltration across tumors [57], while Li *et al.* [58] found that high expression of m7G core genes is associated with poor prognosis in HCC. Additionally, by constructing an immune escape-related protein-protein interaction (PPI) network, they discovered that (*METTL1*, *WDR4*) and 19 mRNA risk signature genes are related to immune escape. This study identifies significant associations between *WDR4* and macrophages in HCC, particularly *TAMs*. Immunohistochemistry confirms significant positive correlations between *WDR4* expression and both PD-L1 and CD68 levels. Notably, PD-L1 expression showed no significant correlation with DFS under postoperative immunotherapy

monotherapy, whereas elevated *WDR4* expression corresponded to shorter DFS in immune monotherapy recipients, suggesting *WDR4* as a potential HCC immunotherapy indicator.

5. Conclusion

WDR4 exhibits elevated expression levels within HCC tissues and is associated with immune infiltration, which establishes it as a prognostic biomarker in HCC. Furthermore, the positive correlation observed between *WDR4*, CD68, and PD-L1 underscores its potential as a guiding factor in immunotherapeutic approaches for HCC.

Availability of Data and Materials

The datasets supporting the findings of this study are available from the corresponding author upon reasonable request. Any further inquiries regarding methods or analysis details will also be provided by the authors upon request.

Author Contributions

SSF and LYY conceived and designed the study. SSF and LYY as well as acquisition and management funds. LYY, SSF, ML, JW, YZ, MM, JJF captured, collected, and extracted the data contained in this analysis. SSF and JJW analysis data. LYY originally drafted the paper. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

In this study, 37 tissue samples (including tumor and adjacent tissues) and clinical data from cancer patients (HCC) who received postoperative anti-PD-1 monotherapy were approved by the Ethics Committee of Hunan Provincial People's Hospital (The First Affiliated Hospital of Hunan Normal University) (Ethics Approval No. 2019-19). This study was conducted in accordance with the guidelines of the Declaration of Helsinki, and informed consent was obtained from all patients. Furthermore, immunohistochemical staining to evaluate the expression of PD-L1 and CD68 in cancer tissues and adjacent tissues within WDR4 has been approved by the Ethics Committee of Hunan Provincial People's Hospital (The First Affiliated Hospital of Hunan Normal University) (Ethics Approval No. 2024-402).

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Conflict of Interest

The authors declare no conflict of interest.

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