Original Article

NF2 is a candidate diagnosis, prognostic, and immunotherapeutic biomarker: a systematic pan-cancer analysis

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Background: Neurofibromin 2 (*NF2*) regulates diverse cellular events such as transcription, translation, ubiquitination, and micro-RNA biosynthesis. Previous evidence revealed that aberrant expression of *NF2* contributes to tumorigenesis in mesothelioma, meningioma, and breast cancer. However, there is no comprehensive pan-cancer analysis to explore *NF2*'s function in cancer diagnosis, prognosis, and immunological prediction.

Methods: By extensive use of data profiles from The Cancer Genome Atlas (TCGA), Genotype-Tissue Expression (GTEx) project, Cancer Cell Line Encyclopedia (CCLE), CIBERSORT, Human Protein Atlas (HPA), and cBioPortal, we employed various bioinformatics methods to explore the role of *NF2* in pan-cancer, including analyzing the association between *NF2* and tumor diagnosis, prognosis, immune cell infiltration, tumor mutational burden (TMB), and microsatellite instability (MSI). Moreover, the co-expression relationship between *NF2* expression with RNA modification genes was also constructed.

Results: Our research indicated that *NF2* was highly expressed in most kinds of tumors. *NF2* showed an early diagnosis value in 13 types of tumors and was significantly associated with the prognosis in most tumors. The results also verified that *NF2* expression was associated with most immune-related cells and signaling pathways in pan-cancer, especially in diffuse large B-cell lymphoma and ovarian serous cystadenocarcinoma. Furthermore, *NF2* gene expression was associated with TMB and MSI in many tumors.

Conclusions: Our study reveals that *NF2* might be helpful in tumor early diagnosis and prognosis evaluation. The expression of *NF2* is highly associated with the tumor immune microenvironment. Additionally, *NF2* is a potential biomarker for predicting the efficacy of immune checkpoint inhibitors therapy. Therefore, *NF2* can be a promising diagnostic, prognostic, and immunotherapeutic biomarker for many types of tumors.

Keywords: Neurofibromin 2 (NF2); pan-cancer analysis; diagnosis; prognosis; immunotherapy

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Introduction

Cancer mortality is rapidly growing in every country of the world. Almost 10 million people die of cancer every year. Unfortunately, there is still no complete cure for cancer (1). Recently, the tumor immune microenvironment (TIME) has been proven essential in tumor progression. Continued detailed analysis of TIME enables the identification of potential biomarkers for clinic benefits, and multiple novel immune checkpoints have evolved into practical cancer therapy targets (2). With the generation and continuous

improvement of public databases like The Cancer Genome Atlas (TCGA), it is possible to explore the occurrence and progression mechanisms of cancer through pan-cancer analysis, which may help to find potential biomarkers and immunotherapeutic targets and provide new insights for diagnosis, treatment, and prognosis of cancer (3).

The human neurofibromin 2 (*NF2*) is a protein-coding gene expressing a protein called Merlin (4). Merlin is a typical tumor suppressor that is known for its ability to induce contact-dependent growth inhibition. Like other multifunctional proteins, merlin plays an important role in maintaining cell stability, controlling cell proliferation, and promoting tissue and organ differentiation by interacting with cell surface proteins, proteins involved in cytoskeletal dynamics, and proteins involved in regulating ion transport (5). *NF2*'s malfunction results in aberrant cell proliferation causing tumorigenesis by abrogating anti-tumor immunity and modulating primary cell proliferation signaling pathways, including Hippo, WNT/β-catenin, TGF-β, receptor tyrosine kinase (RTK), and Notch pathways (6-10). Studies have manifested that mutation of *NF2* is recognized in malignant tumors, such as breast cancer (BRCA), mesothelioma (MESO), prostate cancer, and glioma, and may lead to poor prognosis (5,11). In addition, some clinical trials have been conducted to explore the prognostic impact of *NF2* mutations on solid tumor patients (12,13).

The occurrence of *NF2* mutations can affect tumor progression or prognosis. However, there is still a lack of larger researches to evaluate the inactivation of *NF2* in cancer. We still need to better understand the role of *NF2* in tumor development and progression. The fact that the absence of *NF2* mainly leads to the formation of tumors in Schwann, meningeal, and ependymal cells, while other cell types, although commonly expressing merlin in normal tissues, do not undergo transformation, indicating that tissue specific molecular background and tumor microenvironment dependence need further clarification (7). There is still no comprehensive pan-cancer study of *NF2* conducted. Therefore, we retrieved multiple databases including TCGA, Human Protein Atlas (HPA), Cancer Cell Line Encyclopedia (CCLE), Genotype-Tissue Expression Project (GTEx), and cBioPortal to extract corresponding data for subsequent analysis. With the comparison and analysis of *NF2* expression in various types of tumors, we studied associations between *NF2* with immune infiltration levels, immune-related genes expression, microsatellite instability (MSI), and tumor mutational burden (TMB). Besides, we also investigated gene set enrichment analysis (GSEA) and gene set variation analysis (GSVA) to explore the biological functions of *NF2* in pan-cancer. The results showed that *NF2* might be a valuable diagnostic, prognostic, and immunological biomarker of pan-cancer. The combination of molecular therapies around *NF2* may become a successful treatment method. Through further research and better understanding of *NF2* related molecular cross signaling, may determine the optimal treatment strategy and achieve personalized precision medicine. We present this article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1179/rc).

Highlight box

Key findings

- Neurofibromin 2 (*NF2*) is upregulated in various tumors, and its expression is highly associated with the tumor immune microenvironment. *NF2* also has a potential value for predicting the efficacy of immune checkpoint inhibitors therapy.

What is known and what is new?

- The abnormal expression of *NF2* contributes to tumorigenesis and progression.
- *NF2* is significantly correlated with immune cells and genes.

What is the implication, and what should change now?

- *NF2* can be used as diagnostic, prognostic, and immunotherapeutic biomarker for pan-cancer.

Methods

Differential expression analysis

The gene expression RNA sequencing and phenotype profiles of TCGA were downloaded from Xena (https://xena.ucsc.edu/), an online platform containing private and public clinical/phenotype data, including tumor samples and corresponding normal samples (14). Data from CCLE were downloaded from DepMap Portal (https://depmap.org/portal/). GTEx (https://www.gtexportal.org/home/) is a comprehensive public dataset to study tissue-specific gene expression and regulation (15). We downloaded 31 different tissues' gene expression data from GTEx. The differential expression gene analyses between tumor and normal samples were performed by Log2 transformation and *t*-tests. $P<0.05$ was the standard for identifying the expression difference between tumor and normal tissues. R software (Version 4.0.2; https://www.Rproject.org) was used

for statistical analysis, and the R package "ggplot2" was used for drawing box plots.

HPA

HPA (https://www.proteinatlas.org/) is a human proteome atlas database that maps human proteins by integrating various omics technologies, such as systems biology, antibody-based imaging, and transcriptomics (16). To evaluate differences in *NF2* expression at the protein level, we downloaded and analyzed immunohistochemistry images of normal and tumor tissues in six types of cancer, including cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), pancreatic adenocarcinoma (PAAD), prostate adenocarcinoma (PRAD), liver hepatocellular carcinoma (LIHC), lung squamous cell carcinoma (LUSC), and testicular germ cell tumors (TGCT). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Diagnosis and survival analysis

Data from TCGA samples regarding tumor node metastasis (TNM) stage and clinical phenotype were analyzed by R packages "ggplot2" to find their correlation with *NF2* expression. To assess the accuracy of *NF2* for disease diagnosis, we performed the receiver operating characteristic (ROC) curve analysis using the R package "pROC" (17). Area under the curve (AUC) of ROC was classified into three ranges: high diagnostic accuracy (AUC $\geq$0.9), relative diagnostic accuracy (0.9< AUC $\leq$0.7), and low diagnostic accuracy (0.7< AUC $\leq$0.5). The survival data from TCGA were used to analyze the relationship between *NF2* and prognosis in pan-cancer. Overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI) were considered as the prognosis indicators. The Kaplan-Meier method and log-rank test were used to conduct survival analyses. Survival curves were drawn by the R packages "survival" and "survminer". Furthermore, R packages "Forestplot" were used to perform cox proportional hazards analysis between *NF2* expression and survival.

Immunological correlation analysis

The relative scores of immunocyte abundances were analyzed by CIBERSORT (https://cibersortx.stanford.edu/), a metagene tool to estimate cell abundances through gene expression data. The correlations between *NF2* and immunocytes in pan-cancer were assessed by R packages "ggplot2", "ggpubr", and "ggExtra". Estimation of Stromal and Immune Cells in Malignant Tumor Tissues Using Expression Data (ESTIMATE) algorithm is able to use transcriptional profiles of cancer samples to infer the abundance of tumor cells, immune cells, and stromal cells in the tumor microenvironment (18). The relationship between immune scores and *NF2* expression in each type of cancer was analyzed by R packages "estimate" and "Limma". Furthermore, the co-expression analysis of *NF2* and immune-related genes, including chemokine, immunoinhibitor, immunostimulator, chemokine receptors, and MHC genes was performed. The R package "Limma" was used to perform the analysis.

TMB is a quantitative genomic biomarker that quantifies the total number of mutations in a tumor specimen to evaluate response to immunotherapy (19). MSI is proven to correlate with better survival outcomes (20). TMB and MSI analyses were performed by Sangerbox, a platform that integrated Gene Expression Omnibus, TCGA, International Cancer Genome Consortium, and other databases for differential analysis and customizable interactive analysis (21).

The biological function enrichment analysis

GSEA and GSVA were conducted to explore biological function enrichment analyses of *NF2* in each tumor. Functional analysis was performed by R packages "limma", "org.Hs.eg.db", "clusterProfiler", and "enrichplot". The GSVA gene set "h.all.v7.5.1" was from the MSigDB database (https://www.gsea-msigdb.org/). The correlation of *NF2* expression with the Hallmark pathway in each tumor was analyzed using R package "GSVA".

Alteration and RNA modification analysis

Alterations of *NF2* in pan-cancer were calculated by the cBioPortal platform (http:// www.cbioportal.org/). Then, we extracted the expression of *NF2* as well as 44 genes involved in the three categories of RNA modification (m6A, m5C, m1A) from the TCGA database. After filtering all normal samples, all genes were performed a log2 transformation to each expression value, and next, we calculated correlations between *NF2* and 44 RNA modification genes.

Statistical analysis

The statistical analysis was computed by R (version 4.2.1)

in this study. These results were considered as statistically significant at P<0.05.

Results

Differential expression of NF2 between tumor and normal tissue samples

By using the GTEx datasets, we analyzed the expression levels of *NF2* gene across different physiologic tissues (*Figure 1A*). It can be seen that *NF2* expression level in testis was the highest among all of the tissues, while most other normal tissues expressed low level of *NF2*. The *NF2* expression levels across different cell lines are presented in *Figure 1B*, derived from the CCLE data set. Compared with the GTEx analysis, it is evident that the expression levels of *NF2* are generally increased in different tissues of cancer cell lines.

Next, through the GTEx-TCGA data of Xena, we compared the expression of *NF2* gene in normal tissues and tumor samples (*Figure 1C*). Excluding the cancers with no or few normal samples, the expression of *NF2* in a total of 25 cancers was statistically different from normal tissues. *NF2* was upregulated in BRCA, cervical squamous cell carcinoma and CESC, cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), diffuse large B-cell lymphoma (DLBC), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), kidney renal papillary cell carcinoma (KIRP), acute myeloid leukemia (LAML), brain lower grade glioma (LGG), LIHC, lung adenocarcinoma (LUAD), LUSC, ovarian serous cystadenocarcinoma (OV), PAAD, prostate adenocarcinoma (PRAD), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), thymoma (THYM). Contrary to the upregulated tumors, *NF2* levels had low expression in adrenocortical carcinoma (ACC), glioblastoma multiforme (GBM), kidney renal clear cell carcinoma (KIRC), and TGCT. Besides, *NF2* expressed no significant difference in bladder urothelial carcinoma (BLCA), pheochromocytoma and paraganglioma (PCPG), rectum adenocarcinoma (READ), uterine corpus endometrial carcinoma (UCEC), and uterine carcinosarcoma (UCS). For paired tumors and corresponding normal samples, *NF2* expression was upregulated in 11 types of tumors (*Figure 1D*). These results suggested that *NF2* may have a potentially crucial role in cancer diagnosis.

Furthermore, in order to analyze the expression of *NF2* at protein level, we collected immunohistochemistry images from the HPA dataset and compared the images with the TCGA expression data (*Figure 1E*). The analysis indicated that the results from two databases were consistent.

Prognostic significance of NF2 in pan-cancer

To elucidate the relationship between *NF2* expression level and prognosis, we performed survival association analysis in pan-cancer, including OS, DSS, and PFI. Cox proportional hazards model analysis showed that *NF2* expression levels were associated with OS in ACC (P<0.001), LIHC (P=0.04), KIRC (P=0.0017), KIRP (P=0.009), LGG (P=0.02), PCPG (P=0.04). *NF2* was a low-risk factor in KIRC, KIRP, LGG, and PCPG, while it was a high-risk factor in other types of cancer (*Figure 2A*).

Next, *Figure 2B* reveals the *NF2* expression significantly correlated with DSS in ACC (P<0.001), LIHC (P=0.0056), KIRC (P<0.001), KIRP (P<0.001) and LGG (P=0.01). Regarding associations between *NF2* expression and PFI, forest plots showed associations in ACC (P<0.001), LIHC (P=0.027), BLCA (P=0.0032), UVM (P=0.04), KIRC (P<0.001), KIRP (P=0.0055), LGG (P=0.02) and THYM (P=0.02) (*Figure 2C*).

Kaplan-Meier survival analysis demonstrated that high *NF2* expression was associated with better OS among patients with GBM, KIRC, KIRP, OV, PAAD, and THYM. On the contrary, in ACC, sarcoma (SARC), SKCM and TGCT, high expression of *NF2* was associated with poor OS (*Figure 2D-2M*). In addition, Kaplan-Meier survival analysis suggested the correlation between low *NF2* expression level and better DSS in ACC, SARC, and SKCM. At the same time, patients in PAAD, GBM, KIRC, MESO, KIRP, THYM, and STAD showed the opposite (Figure S1). Low expression of *NF2* was associated with poor PFI in patients with ESCA, GBM, KIRC, KIRP, and LGG (Figure S2).

Diagnosis value of NF2 in pan-cancer

By analyzing the expression of *NF2* in different stages of tumors, we found that the expression of *NF2* increased significantly in the early stages of 9 cancers, including CHOL, COAD, HNSC, LIHC, LUSC, KIRP, oral squamous cell carcinoma (OSCC), PRAD and STAD (*Figure 3A*). The analysis indicated that *NF2* might have a potential clinical value in the early diagnosis of the tumors mentioned above. Furthermore, the ROC curve demonstrated the

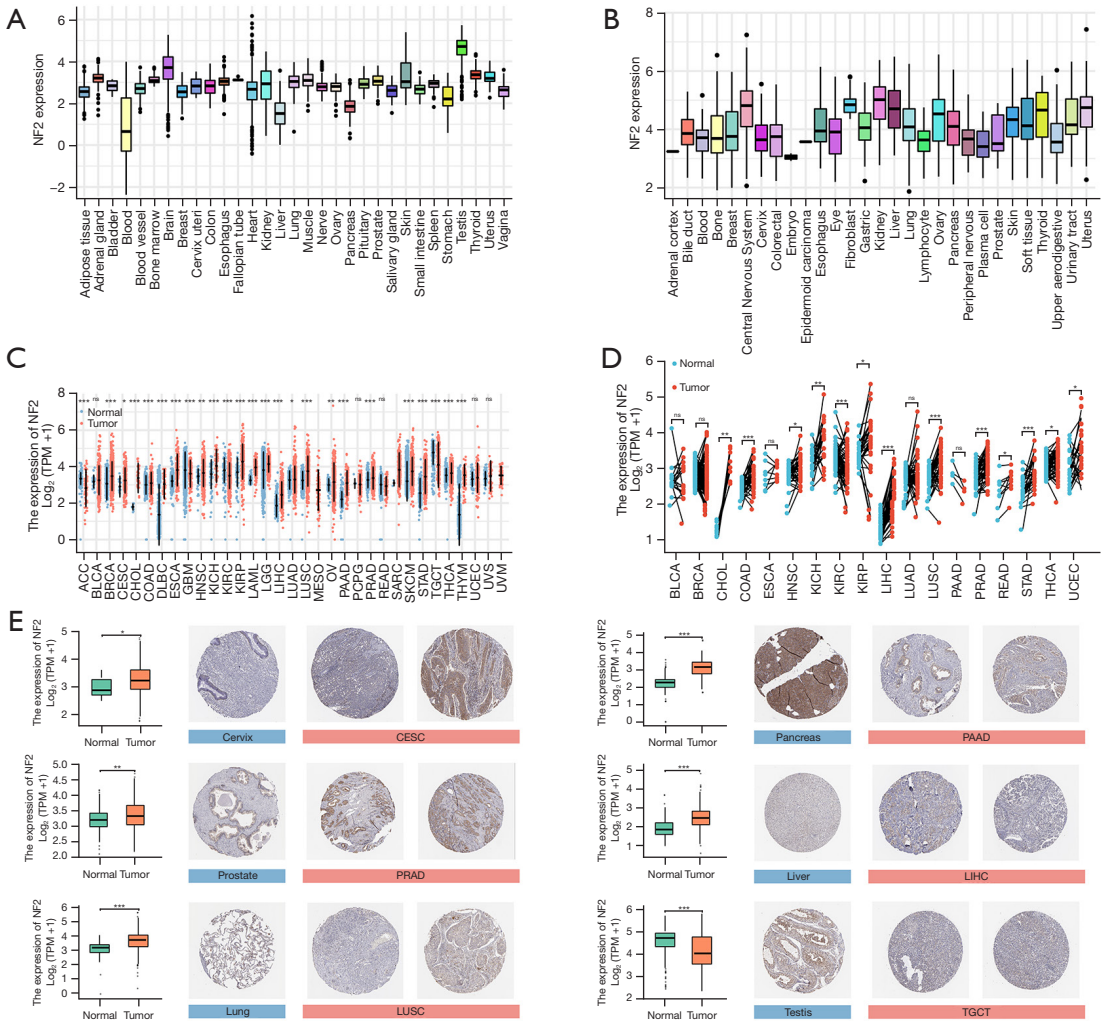

Figure 1 Differential expression of *NF2*. (A) *NF2* expression in normal tissues. (B) *NF2* expression in tumor cell lines. (C) Expression of *NF2* in 33 types of tumors. (D) Comparison of *NF2* expression in paired tumor and normal samples. (E) Comparison of *NF2* gene expression between normal and tumor samples (left) and immunohistochemistry images in normal (middle) and tumor (right) tissues (×200). Tissue images available from https://www.proteinatlas.org/ENSG00000186575-NF2/tissue. Tumor images available from https://www.proteinatlas.org/ENSG00000186575-NF2/pathology/cervical+cancer (CESC); https://www.proteinatlas.org/ENSG00000186575-NF2/pathology/pancreatic+cancer (PAAD); https://www.proteinatlas.org/ENSG00000186575-NF2/pathology/prostate+cancer (PRAD); https://www.proteinatlas.org/ENSG00000186575-NF2/pathology/liver+cancer (LIHC); https://www.proteinatlas.org/ENSG00000186575-NF2/pathology/lung+cancer/LUSC (LUSC); https://www.proteinatlas.org/ENSG00000186575-NF2/pathology/testis+cancer (TGCT). *, P<0.05; **, P<0.01; ***, P<0.001; ns, no significance. *NF2*, neurofibromin 2; TPM, transcripts per kilobase per million mapped reads; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; 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 serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma, TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

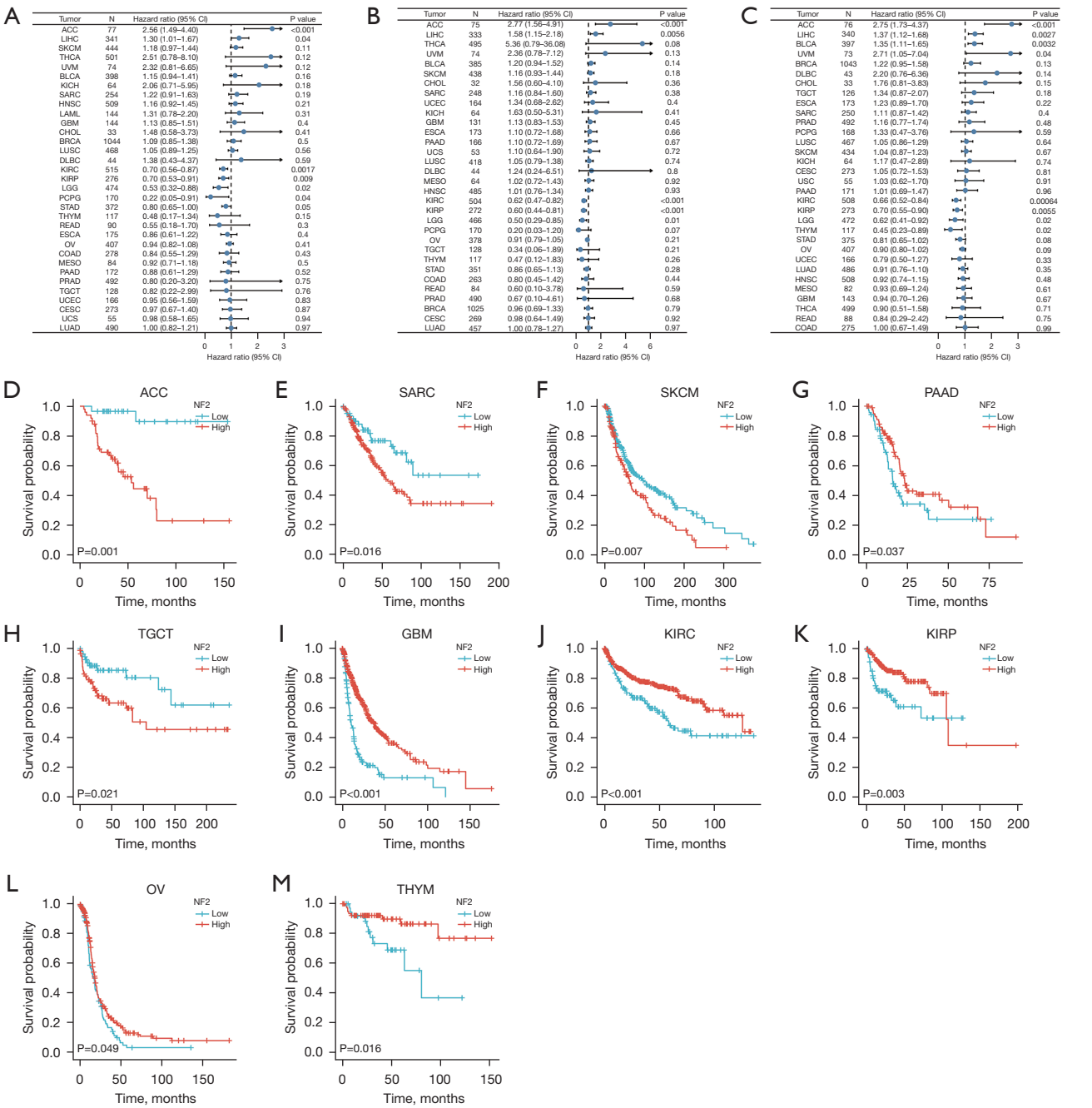

Figure 2 Tumor survival analysis for *NF2* in pan-cancer. (A) Association between *NF2* expression and OS. (B) Association between *NF2* expression levels and DSS. (C) Association between *NF2* expression levels and PFI. Forest plot of OS associations in 33 types of tumors. (D-M) Kaplan-Meier analysis of the association between *NF2* expression and OS. CI, confidence interval; *NF2*, neurofibromin 2; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval.

performance of the gene signature for diagnostic accuracy. Results were divided into three groups: high diagnostic accuracy with the AUC >0.9, relative diagnostic accuracy (0.9< AUC <0.7), or low diagnostic accuracy (0.7< AUC <0.5). As *Figure 3B* shows, there were 4 types of cancer with high diagnostic accuracy, 9 types with relative diagnostic accuracy 14 types with low diagnostic accuracy. By the way, *NF2* expression had the best diagnostic accuracy in CHOL,

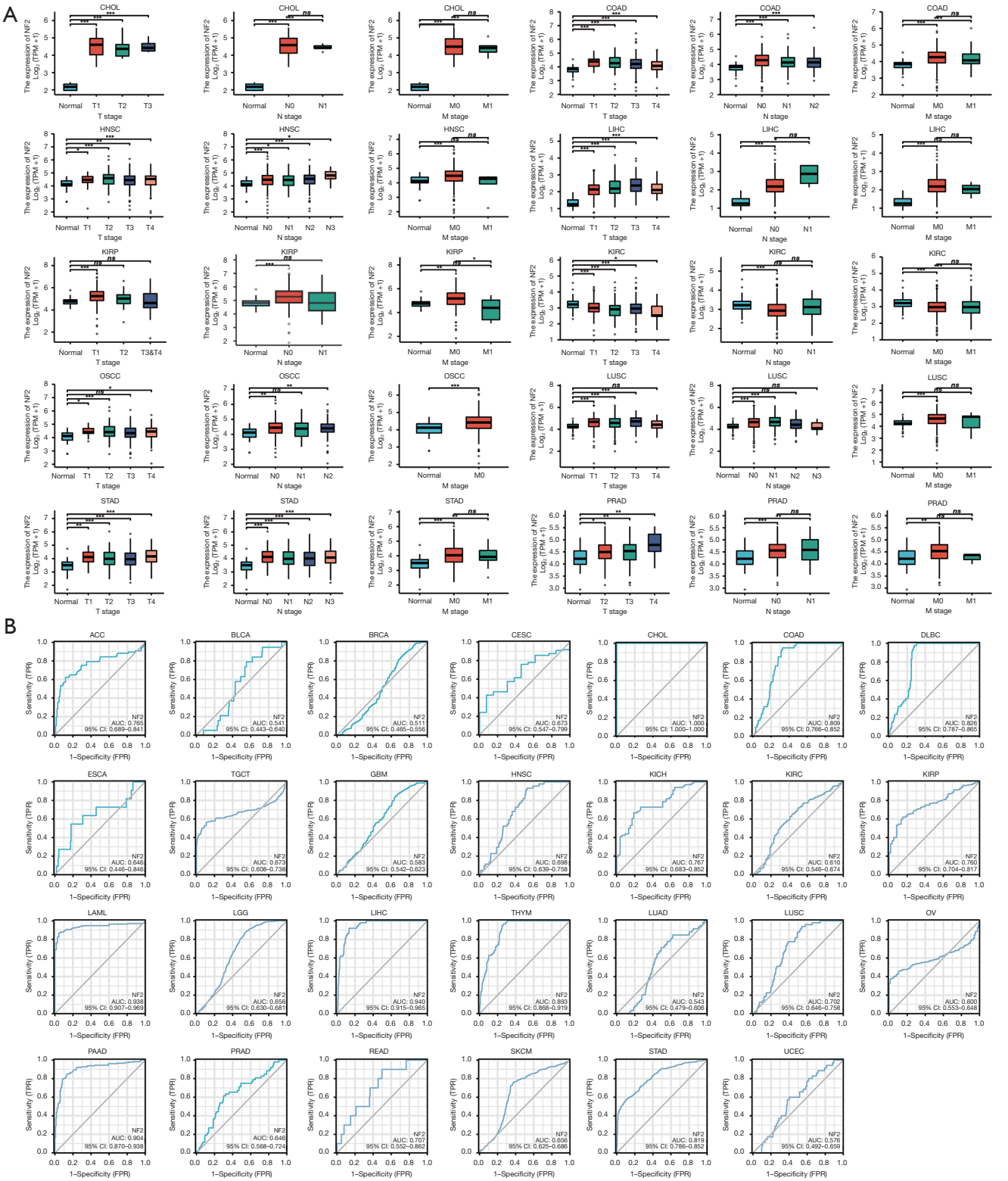

Figure 3 Diagnosis analysis of *NF2* in pan-cancer. (A) Association between *NF2* gene expression and tumor node metastasis stage. *, P<0.05; **, P<0.01; ***, P<0.001; ns, not statistically significant. (B) Area under curve of receiver operating characteristic curves verified the diagnosis performance of *NF2*. TPM, transcript per million; *NF2*, neurofibromin 2; TPR, true positive rate; FPR, false positive rate; AUC, area under the curve; CI, confidence interval.

with 1.0 AUC achieved.

Relationship between NF2 expression levels and tumor immune infiltration

Tumor immune cell infiltration is a critical factor in tumor progression and immune escape. The analysis by CIBERSORT revealed the correlation between *NF2* expression and immune cell infiltration. The results showed that *NF2* expression level had a significant association with immune cell infiltration of a variety of cancers. In particular, *NF2* expression was associated with Th2 cells in 19 types of cancer, pDc cells in 18 types, and cytotoxic cells in 16 types (*Figure 4A*). We further explored the relationship between TIME and *NF2* expression by the ESTIMATE algorithm. The immune cell scores were calculated in 33 types of tumors. The results revealed that *NF2* expression was significantly negatively correlated with immune score in GBM, LGG, CESC, BRCA, ACC, ESCA, SARC, PRAD, UCEC, KIRC, LUSC, THYM, THCA, and SKCM, and contrarily in PAAD, COAD, DLBC, and UVM (*Figure 4B*).

Relationship between NF2 expression with immune-related genes

In order to explore the relationship between *NF2* expression and immune-related genes, gene co-expression analyses were performed between *NF2* expression and immune-related genes, including chemokines, chemokine receptors, immunostimulators, immunoinhibitors, and major histocompatibility complex (MHC) genes (*Figure 5A-5E*). It can be seen that *NF2* was positively correlated with the expression of most immune-related genes, especially with VEGFR, TGFBR1, IL10RB, TAP1, TAP2, CD276, PVR, and IL6R. In addition, immune-related genes significantly correlated with *NF2* expression in DLBC, OV, PAAD, and UVM.

NF2 mutations and correlation of NF2 with RNA modification genes

The gene alteration of *NF2* in pan-cancer was analyzed through the cBioPortal platform. The TCGA pan-cancer atlas studies, covering 10,967 patients from 32 types of cancer, were used for gene alteration analysis. cBioPortal revealed that mutation was the largest proportion of alteration in *NF2* and occurred most frequently in pleural MESO (22.99%), CHOL (5.56%), and endometrial cancer (3.75%) (*Figure 6A*). The integration data of the *NF2* mutation sites are shown in *Figure 6B*. Besides, we further analyzed the correlation between *NF2* expression and RNA modification genes, including m6A/m1A/m5C regulated genes (*Figure 6C-6E*). The result indicated that the expression of *NF2* was positively correlated with most m6A/m1A/m5C regulated genes, especially in DLBC and OV.

Biological functions analysis of NF2 in pan-cancer

To investigate the potential biological pathways correlated with *NF2*, we conducted GSEA and GSVA in pan-cancer. The visualized results of GO and KEGG pathway analysis are shown in *Figure 7A*. The enriched pathways mainly focused on the mechanism of immune regulation, such as regulation of lymphocyte activation (BLCA, DLBC, COAD, LIHC), immune response regulation signaling pathway (DLBC, GBM, LIHC), and intestinal immune network for immunoglobulin A production (COAD). Besides, *NF2* was also closely associated with MAPK signaling pathways, pathways in cancer, and neuroactive ligand-receptor interaction in various tumors. For GSVA results, the top 5 hallmark pathways significantly positively and negatively associated with *NF2* expression in various tumors are presented in *Figure 7B*. *NF2* expression has the highest positive correlation with WNT/β-catenin pathway and the highest negative correlation with interferon-α response. In summary, these results suggested that *NF2* may play a critical role in tumor immunity regulation, tumorigenesis, and tumor progression.

TMB and MSI analysis of NF2 expression in pan-cancer

TMB and MSI are closely related to the sensitivity of immune checkpoint inhibitors (ICIs), such as programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1). By Spearman rank correlation coefficient, we calculated the TMB and MSI of each tumor sample and analyzed the correlation between *NF2* with TMB and MSI in 33 tumors. The results revealed that *NF2* was positively correlated with TMB in ACC, BRCA, COAD, LAML, SARC, STAD, UCEC, and conversely in THCA and UVM (*Figure 8A*). *NF2* expression was positively correlated with MSI in 7 tumors, including BLCA, CESC, COAD, KICH, SARC, STAD, and UCEC. Additionally, *NF2* was negatively correlated with MSI in DLBC, HNSC, PRAD, and THCA (*Figure 8B*). The above results showed that high expression of *NF2* is extensively associated with tumor immunity.

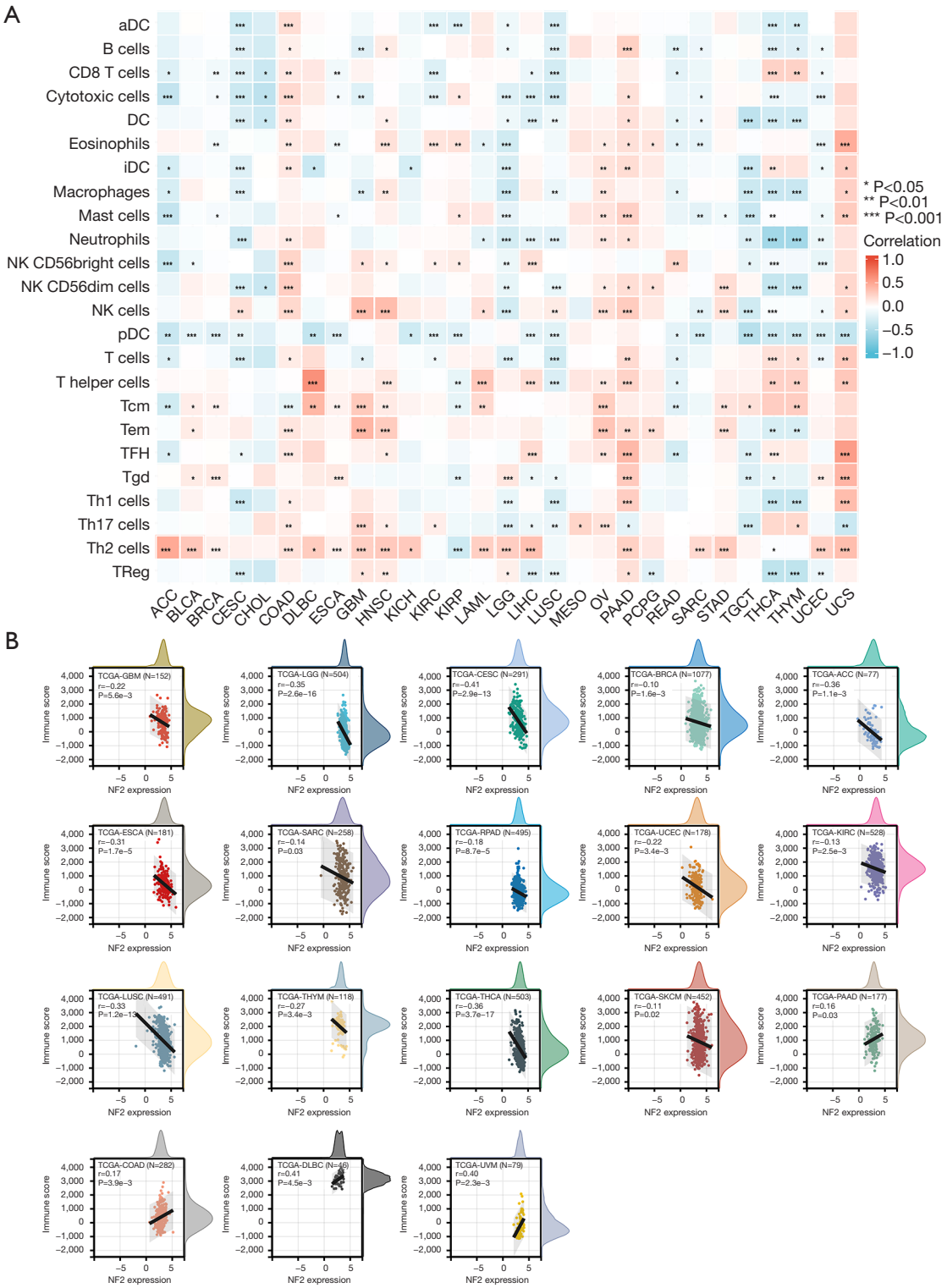

Figure 4 Relationship between *NF2* and tumor immune infiltration. (A) Correlation between *NF2* expression and tumor infiltration levels across different immune cells. (B) Relationship between *NF2* expression and immune score calculated by ESTIMATE. TCGA, The Cancer Genome Atlas; *NF2*, neurofibromin 2.

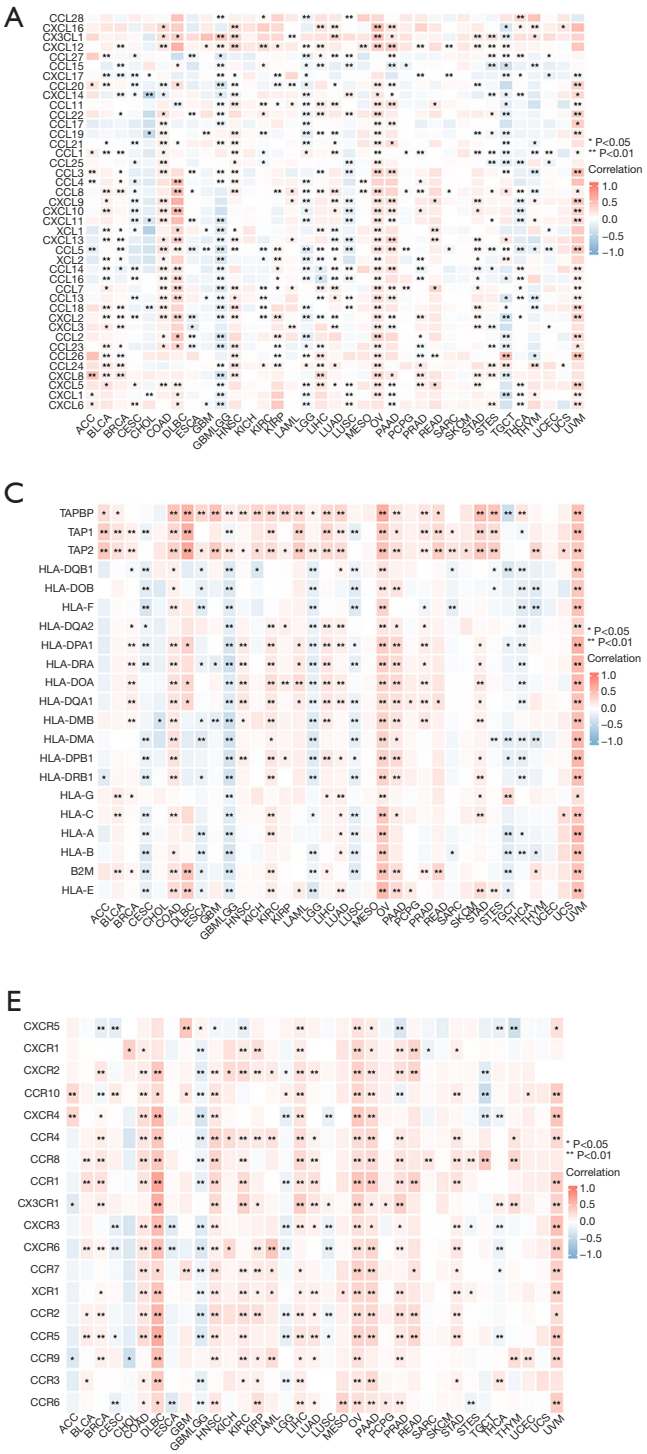

Figure 5 Correlation between *NF2* expression and that of immune-related genes. (A) Chemokine genes. (B) Immunoinhibitor. (C) MHC gene. (D) Immunostimulator. (E) Chemokine receptors. *NF2*, neurofibromin 2; MHC, major histocompatibility complex.

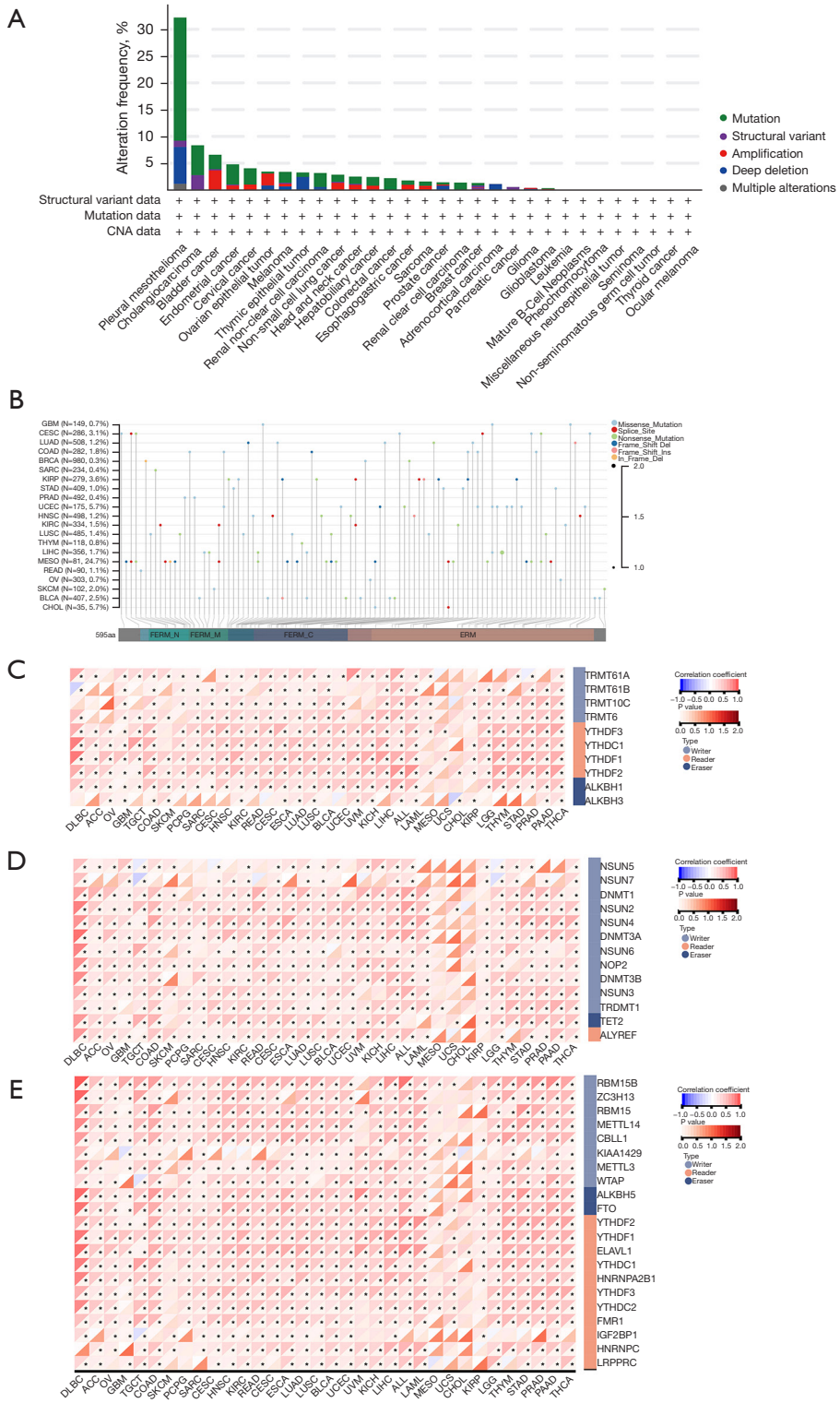

Figure 6 Genetic alterations and RNA modifications of *NF2*. (A) Alteration frequency of *NF2* from cBioPortal; (B) mutation sites of *NF2* in pan-cancer; (C) co-expression between *NF2* and m1a genes; (D) co-expression between *NF2* and m5c genes; (E) co-expression between *NF2* m6a genes. *, P<0.05. CNA, copy number alteration; *NF2*, neurofibromin 2; FERM, four-point-one/ezrin/radixin/moesin; ERM, ezrin/moesin/moesin.

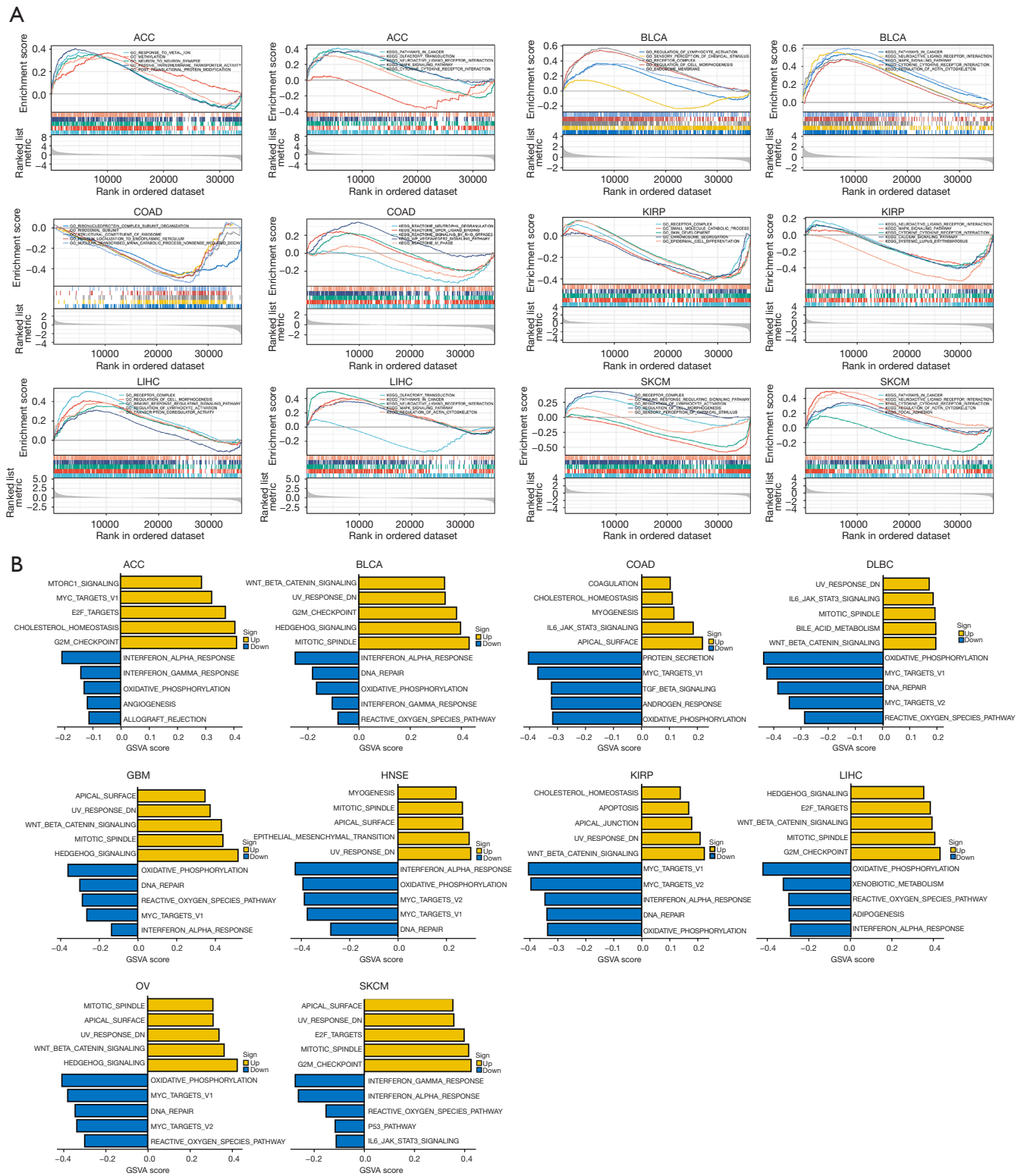

Figure 7 Biological functions analysis of *NF2*. (A) GSEA of *NF2* in various tumors. (B) GSVA of *NF2* in TCGA in several tumor types. GSVA, gene set variation analysis; *NF2*, neurofibromin 2; GSEA, gene set enrichment analysis; TCGA, The Cancer Genome Atlas.

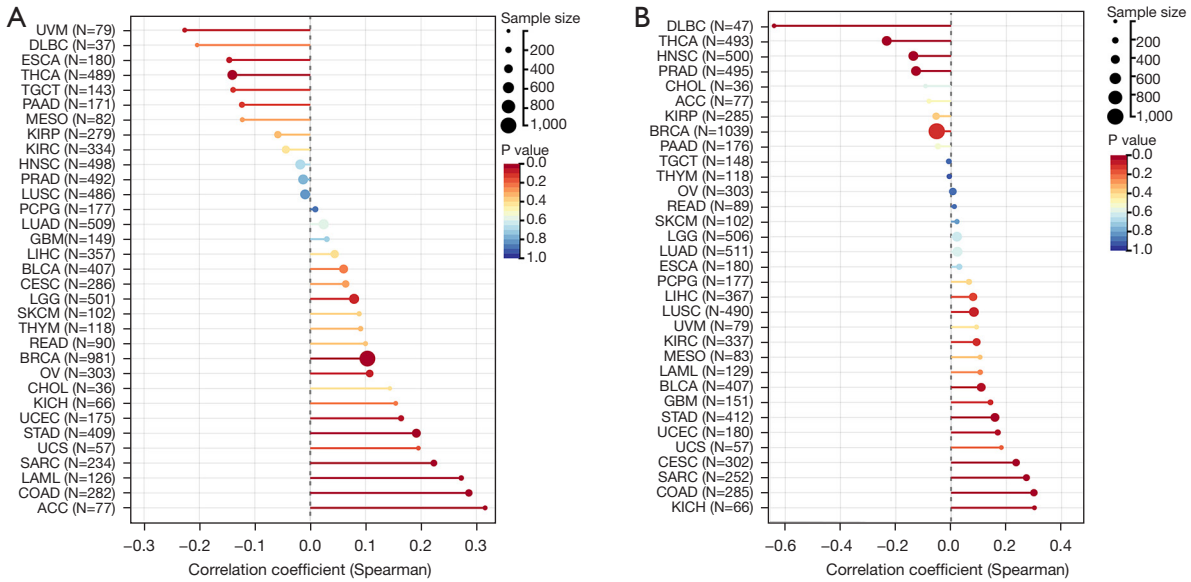

Figure 8 Associations between *NF2* expression and TMB, MSI. (A) The relationship between *NF2* and TMB. (B) The relationship between *NF2* and MSI. *NF2*, neurofibromin 2; TMB, tumor mutational burden; MSI, microsatellite instability.

Discussion

The activation of developmental signaling pathways is a similarity between embryonic tissue growth and tumorigenesis. WNT/β, TGF-β, RTK, Hippo, and Notch pathways are key participants in normal developmental biology (22). Loss of restriction on developmental related signaling pathways can cause damage to tissue development, manifested as developmental syndrome. Similarly, by promoting cell proliferation, migration, and stem cell like phenotype, the activity imbalance of these pathways can promote cancer occurrence and progression. As a spatiotemporal-dependent manner, *NF2* contributes to either activation or inhibition of developmental pathways in order to maintain cell integrity, tissue organization, and adequate different stages of organism development. The abnormality of *NF2* deregulates the activity of these pathways and promotes carcinogenesis and cancer progression. However, *NF2* has not received much attention in cancers (11). In our study, there are significant differences in *NF2* expression of 25 cancer types compared with normal tissues. Among them, *NF2* expression levels were upregulated in 21 tumors, such as BRCA, CESC, CHOL, and COAD. Although some cancer analyses could not be conducted due to the lack of normal tissue data, they are still worth further exploration with accumulating datasets. For example, genetic inactivation of *NF2* was confirmed as a frequent tumorigenic event in MESO (23). In paired samples analysis, *NF2* was overexpressed in 11 cancer types, and IHC analysis confirmed the high-level expression of *NF2* in most cancer types at the protein level. These results indicate the potential of *NF2* as a tumor biomarker. To investigate the association between *NF2* expression levels and prognosis, survival association analysis was performed using Kaplan-Meier survival curves for each type of cancer, including OS, DSS, and PFI. Combining these results, we found that high *NF2* expression had a good prognosis in ESCA, LGG, PAAD, GBM, KIRC, MESO, KIRP, THYM, STAD, OV, and a poor prognosis in ACC, CHOL, LIHC, SARC, SKCM, and TGCT. Given the above, we also investigated the expression of *NF2* in different TNM stages to explore the value of *NF2* in early cancer screening. Based on the collected data marked with staging information, we found that *NF2* expression had early elevations in 9 cancers. Besides, *NF2* also showed a superior diagnosis value in the AUC of ROC curve. *NF2* showed high diagnostic accuracy in 4 forms of cancer (AUC ≥0.9), including COAD, with 1.0 AUC achieved. Nine forms of cancer showed a relative diagnostic accuracy (0.9< AUC ≤0.7). To understand the relationship between *NF2* expression level and cancer prognosis, we performed Cox proportional hazards model analysis and Kaplan-Meier survival curves, including OS, PFI, and DSS. All combined, high *NF2* expression had a better prognosis in GBM, KIRC, KIRP, MESO, THYM,

STAD, OV, ESCA, and LGG, and a worse prognosis in ACC, SARC, SKCM, BLCA, and CHOL. Overall, these findings suggest that *NF2* has potential value in early diagnosing and assessing prognosis.

The occurrence and progression of cancer are closely related to its surrounding stroma (24). Cancer cells and inflammatory cells with their surrounding stroma constitute the TIME. Cells within the TIME are highly plastic, continuously changing their phenotypic and biological functions (25). Growing evidence suggests that TIME can be exploited to assess the response of tumor cells to immunotherapies (26). So, it is essential to understand the TIME status of patients to select the appropriate immunotherapy strategy. In the present study, we found that high expression of *NF2* is associated with high expression of TH2 cells and the inhibition of pDC cells. In addition, in COAD, OV, and PAAD, high expression of *NF2* is correlated with high expression of various immune cells. We also analyzed the correlation between *NF2* expression and immune score calculated by the ESTIMATE algorithm. The results indicate that *NF2* expression negatively correlates with immune cell infiltration in most cancers. The co-expression analysis unraveled a positive correlation between *NF2* and immune-related genes, such as chemokines, immunosuppressive genes, immunostimulatory genes, MHC genes, and their receptors in most tumor types. We found that *NF2* correlated with T cell exhaustion marker genes such as VEGFR, TGFBR1, KDR, IL-10RB, and PDCD1LG2 in pan-cancer (27-29). Previous studies prove that *NF2* can affect the TGF-β signaling pathway, which regulates Treg cells, effector T cells, NK cells, macrophages, and multiple immune response processes (30). VEGFR plays a crucial role in tumor neo-angiogenesis and induces immunosuppression by modulating Treg cells, dendritic cells, cytotoxic T lymphocytes, and M2-like macrophages, resulting in tumor immune escape (31).

Additionally, our GSEA and GSVA results demonstrated the correlation of *NF2* expression with immune regulatory functions, such as lymphocyte activation, immune response pathway, MAPK pathway, and WNT/β-catenin pathway. MAPK pathway has been shown to regulate inflammatory responses by inducing the expression of multiple cytokines, including interleukins and interferons (32-34). Wnt/β-catenin signaling pathway has been proven to regulate the differentiation and development of various immune cells, such as macrophages and B cells (35). In summary, these results indicate that *NF2* expression level is involved in the regulation and activation of immune cells and is highly correlated with the TIME.

ICIs, such as PD-1/PD-L1 inhibitors and CTLA-4 inhibitors, have demonstrated lasting anti-tumor effects in the treatment of multiple cancer types (36). However, only a tiny percentage of patients could have long-term clinical benefits from ICI therapies, and ICIs may cause immune toxicities or even worsen tumor prognosis (37). TMB is a predictive biomarker for identifying patients with better survival upon ICI treatment (38). *NF2* has a significantly positive correlation with TMB in ACC, BRCA, COAD, LAML, SARC, STAD, and UCEC. MSI is also a predictive biomarker of ICIs (39), and it has been confirmed as an independent predictor of clinical characteristics and prognosis in COAD (40). *NF2* positively correlates with MSI in BLCA, CESC, COAD, KICH, SARC, STAD, and UCEC. Based on existing research and our findings, we speculated that patients with high *NF2* expression might benefit from ICIs therapy in the cancers mentioned above.

RNA modification is a critical method of regulating gene expression at post-transcription (41). Aberrant RNA modifications promote the activation of multiple cancer phenotypes, such as stress adaptation, differentiation, invasion, and resistance to therapies (42). In our study, the results suggest a positive correlation between *NF2* expression and major RNA modification genes of m6A, m5A, and m1C, such as METTL3, METTL14, and NSUN2. METTL3 and METTL14 complex was demonstrated as critical factor of cell proliferation (43). NSUN2 has been reported to be able to modulate MYC-dependent proliferation and stabilize oncogenic mRNAs (44,45). These results indicated that *NF2* expression could affect RNA modification and may broaden ideas for anticancer epigenetic drugs.

In brief, our first pan-cancer analysis of *NF2* confirms the differential expression of *NF2* between tumor and normal tissues, and *NF2* expression is correlated with TIME and clinical prognosis. Our findings identify *NF2* as a potential early diagnostic biomarker and independent prognostic factor in pan-cancer. Different expression levels of *NF2* will contribute to different prognostic outcomes, which still need further targeted analyses of *NF2* in each type of cancer. Besides, the expression of *NF2* is associated with TMB and MSI in a variety of cancers, suggesting that *NF2* may be a potential biomarker for predicting the efficacy of ICI therapy.

However, this study has some limitations, which cannot be ignored. First, specific systematic biases may exist because the data used for analysis were derived from different databases (46). Second, although we found that the

expression of *NF2* is related to tumor diagnosis, diagnostic, and immunity, we could not prove the causal relationship. In addition, we found that even as a tumor suppressor, some patients still have poor prognosis when *NF2* is highly expressed. This may be due to the heterogeneity and complexity of tumor occurrence and development, as well as the imbalance and crosstalk between various signaling pathways. However, since our research mainly focuses on bioinformatics analysis methods, we can only analyze whether there is a correlation between the expression levels of genes or proteins, but cannot judge whether there is a causal relationship between them. In the next work, we will continue to increase the in-depth study on the function of *NF2* in the occurrence and metastasis of different cancers and conduct functional experiments on *NF2*.

Conclusions

In summary, *NF2* is related to early diagnosis and prognosis of cancer patients and the immune infiltration in different cancers. The expression of *NF2* is also associated with MSI, TMB, and RNA methylation genes in various cancers. Our study reveals that *NF2* is a promising diagnosis, prognostic, and immunotherapeutic biomarker for many types of tumors. These findings may help elucidate the biological functions of *NF2* in tumorigenesis and progression, ultimately impacting precision medicine and personalized immunotherapy in the future.

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Footnote

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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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