From expression to immune responses: exploring the multifaceted roles of CTNNBIP1 in osteosarcoma

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Abstract

Introduction: The CTNNBIP1 gene has been reported to be involved in development, tumorigenesis, tissue differentiation, and other processes. However, the definite roles of CTNNBIP1 in osteosarcoma remained unclear. Therefore, this study was conducted to explore the roles of CTNNBIP1 in osteosarcoma.
Material and methods: All data for bioinformatics analysis were acquired from GEO and TARGET osteosarcoma datasets. Survival analysis, expression analysis, Cox regression analyses, nomogram, gene set enrichment analysis (GSEA), and immune evaluations were performed sequentially. Experiments of qRT-PCR, western blot and immunohistochemistry were used to verify the expression of CTNNBIP1 in osteosarcoma.
Results: The expression of CTNNBIP1 was higher in osteosarcoma than in normal tissues, and further *in vitro* and *in vivo* experimental results of qRT-PCR, western blot, and immunohistochemistry remained consistent (all $p < 0.05$). Both univariate and multivariate Cox regression analyses revealed that disease metastasis status at diagnosis and CTNNBIP1 were independent predictors of OS in osteosarcoma (both $p < 0.05$). GSEA results indicated that CTNNBIP1 was significantly enriched in three pathways, including B cell receptor and nitrogen metabolism pathways, in osteosarcoma. As for the immunologic roles of CTNNBIP1 in osteosarcoma, CTNNBIP1 was found to be markedly associated with infiltration levels of naïve B cells and resting mast cells and tumor microenvironment (all $p < 0.05$). Furthermore, the TIDE algorithm indicated that osteosarcoma patients with elevated CTNNBIP1 expression have a better immune response to immunotherapy.
Conclusions: Our study indicated for the first time that CTNNBIP1 may serve as a potential biomarker of prognosis and immunotherapy for osteosarcoma.

Key words: CTNNBIP1, biomarker, immunity, osteosarcoma, prognosis.

Introduction

Osteosarcoma, a common malignant bone tumor of children and adolescents, was estimated to have an incidence of 0.5 cases per 100,000 persons per year [1]. This disease, characterized by frequent recurrence and distant metastasis, more often occurs in long bones and is located at the metaphysis [2, 3]. The major cause of death in osteosarcoma was reported to be

lung metastasis, and about 15–20% of these osteosarcoma patients were initially diagnosed with this type of metastasis, along with a depressed survival prognosis [4]. Although there have been great developments in surgery, chemotherapy, radiotherapy, targeted therapy, and immunotherapy, the 5-year survival rates of osteosarcoma sufferers with metastasis or recurrence are often less than 20% [5]. There is still an urgent need to reveal the molecular mechanisms underlying osteosarcoma metastasis or recurrence and to find novel therapeutic targets for future cancer precision medicine.

Catenin beta interacting protein 1 (CTNNBIP1), located at 1p36, has a gene length of 68 kb and was reported to be an antagonist of the Wnt pathways [6]. By competing with T-cell factor for binding to beta-catenin, CTNNBIP1 can inhibit the activities of the beta-catenin/T-cell factor complex, thus affecting various genes in the Wnt/beta-catenin signaling pathway [7]. The CTNNBIP1 gene has been reported to have roles in various processes including development, tumorigenesis, and tissue differentiation [8, 9]. Qu *et al.* demonstrated the vital roles of the CTNNBIP1-CLSTN1 fusion transcript in human neocortical development [10]. Zhang *et al.* shed light on how miR-486-3p could promote the bone marrow mesenchymal stem cells' osteogenic differentiation via targeting CTNNBIP1 and then activating the Wnt/beta-catenin signaling pathway [11]. In lung cancer, Chang *et al.* suggested that the CTNNBIP1 gene could suppress cancer migration and serve as a prognostic biomarker [12]. In a CTNNBIP1-dependent way, over-expressed circRNA_102171 was able to promote the progression of papillary thyroid cancer via the Wnt/beta-catenin signaling pathway [13]. Down-regulated CTNNBIP1 expression was also reported to be associated with viral infections as well as tumor grade in gastric cancer [14]. In osteosarcoma, Rothzerg *et al.* identified 12 survival-based differentially expressed genes, and Yang *et al.* constructed a 17-gene signature, both of which included CTNNBIP1, as novel biomarkers for osteosarcoma [15, 16]. The aforementioned information suggested the importance of CTNNBIP1 in cancer. However, the definite roles of CTNNBIP1 in osteosarcoma remained unclear. Therefore, this study was conducted to explore the prognostic roles of the CTNNBIP1 gene in osteosarcoma and shed light on its relationships with immunity, with the assistance of in vitro and in vivo experiments, providing reliable predictors for future cancer precision medicine.

Material and methods

Gene filtration and single gene expression matrix analysis

Three osteosarcoma-related datasets were included in this study for gene filtration, including the GSE16088 osteosarcoma tissue dataset (tumor = 14; normal control = 3), the GSE33382 osteosarcoma tissue dataset (tumor = 84; normal control = 3) and the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) osteosarcoma tissue dataset (tumor = 86). Intersected genes were acquired from GSE16088 differentially expressed genes, GSE33382 differentially expressed genes, and TARGET osteosarcoma dataset overall survival (OS) significant genes, with a threshold of p-values < 0.05. Finally, six hub biomarkers, including the CTNNBIP1 gene, were identified. We obtained the CTNNBIP1 single gene expression matrix, as well as related clinical information, in the TARGET osteosarcoma dataset for further data analysis.

Bioinformatics analysis by R 4.1.1 software

To reveal the prognostic roles of CTNNBIP1 in osteosarcoma, difference analysis was conducted using the R "limma" package and survival analysis was analyzed using the Kaplan–Meier (K-M) method, according to the medium expression of CTNNBIP1. Cox regression analyses, both univariate and multivariate, were applied to identify independent prognostic predictors of OS from several types of clinical information (primary tumor site, disease metastasis status at diagnosis, race, gender and CTNNBIP1) in osteosarcoma. A nomogram was also constructed to intuitively display the OS prognosis of osteosarcoma sufferers, with the assistance of independent prognostic predictors (disease metastasis status at diagnosis and the CTNNBIP1 gene). Its performances were evaluated by ROC curves, the C-index, and calibration plots. Gene set enrichment analysis (GSEA) was also applied to obtain significantly CTNNBIP1-enriched pathways in osteosarcoma [17, 18].

To understand the immunologic roles of CTNNBIP1 in osteosarcoma, two aspects of immunity–tumor microenvironment and immune cell infiltrations–were further analyzed according to CTNNBIP1 gene expression. Therein, the tumor microenvironment included stromal, ESTIMATE, and immune scores. They were all analyzed by the "ESTIMATE" algorithm and assessed for differences in high-CTNNBIP1 and low-CTNNBIP1 groups [19, 20]. Immune cell infiltrations included the calculations of the expression of 22 types of immune cells. They were all analyzed by the "CIBERSORT" algorithm and assessed for differences among high-CTNNBIP1 and low-CTNNBIP1 groups [21, 22]. The Tumor Immune Dysfunction and Exclusion (TIDE) dataset was used to predict the immune responses to immunotherapy in high-CTNNBIP1 and low-CTNNBIP1 groups [23, 24]. We also applied the Cellminer dataset (https://discover.nci.nih.gov/cellminer/home.do) to obtain CTNNBIP1-significantly associated drugs for future related drug development [25].

Real-time quantitative reverse transcription PCR (qRT-PCR)

In vitro experiments were conducted in osteosarcoma cell lines, purchased from Procell (Wuhan, China), including MG-63, SW1353, 143B, and one normal control cell line, hFOB. TRIzol reagent (Invitrogen) was used to extract total RNA, and cDNA was obtained by the PrimeScript RT-PCR kit (TaKaRa). Then, qRT-PCR was conducted to verify the mRNA expression levels in osteosarcoma cell lines using the ABI QuantStudio5 Real-Time PCR System. Related primers were as follows: human-CTNNBIP1-forward: 5′-GGCGGCACCTTCCTACTTC-3′, human-CTNNBIP1-reverse: 5′-GCTGTCAGGTTTGATCCCATC-3′; human-ACTB-forward: 5′-TAGTTGCGTTACACCCTTTCTTG-3′, human-ACTB-reverse 5′-TGCTGTCACCTTCACCGTTC-3'.

Western blot

Total protein was extracted from osteosarcoma cell lines (MG-63, SW1353, 143B) and the normal control hFOB, and protein concentration was measured by the BCA method according to the manufacturer's instructions [26, 27]. Equal amounts of protein (30 µg per sample) were separated by 10% SDS-PAGE and transferred to PVDF membranes. The membranes were blocked with 5% non-fat milk for 1 h and incubated overnight at 4°C with primary antibodies (CTNNBIP1, Abcam, ab129011, 1 : 1000). The prestained protein marker was purchased from Servicebio (G2058-250UL). After incubation with HRP-conjugated secondary antibodies for 1 h, bands were visualized by ECL and quantified using ImageJ.

Immunohistochemistry

As described in previous articles [28, 29], formalin-fixed, paraffin-embedded tissue sections (4 µm) from osteosarcoma patients were deparaffinized, rehydrated, and subjected to antigen retrieval in citrate buffer (pH 6.0). Endogenous peroxidase was blocked with 3% hydrogen peroxide, followed by blocking with 10% goat serum. Sections were incubated overnight at 4°C with primary antibody (CTNNBIP1, Abcam, ab129011, 1 : 200), and staining was visualized using DAB after incubation with secondary antibodies. Results were evaluated by two independent pathologists. This research was approved by the Institutional Research Ethics Committees of Affiliated Maternity and Child Healthcare Hospital of Nantong University.

Statistical analysis

All bioinformatics analyses were performed using R 4.1.1 software. Quantitative analyses of qRT-PCR and Western blot were conducted using GraphPad Prism 7 (GraphPad Software Inc.). OS was selected as the clinical outcome of this study. Student's *t*-test was used to compare two groups, and the one-way analysis of variance test was used to evaluate variance among three or more groups. *P*-values were adopted as two-sided, and values below 0.05 were regarded as indicating statistical significance.

Results

CTNNBIP1 mRNA and protein expression in osteosarcoma

The Venn diagram showed that intersected genes were acquired from GSE16088 differentially expressed genes, GSE33382 differentially expressed genes, and TARGET osteosarcoma dataset overall survival (OS) significant genes, with *p*-values < 0.05. Finally, six hub biomarkers, including the CTNNBIP1 gene, were identified (Figure 1 A). Boxplots from GSE16088 and GSE33382 showed that its levels were higher in osteosarcoma tissues than in normal controls (both $p < 0.05$; Figures 1 B, C). As shown in Figure 1 D, high CTNNBIP1 expression was correlated with increased OS ($p < 0.05$). *In vitro* experiments were conducted in osteosarcoma cell lines by qRT-PCR and western blot, and they both indicated elevated expression of CTNNBIP1 in osteosarcoma cell lines (MG-63, SW1353, 143B) compared to the normal control cell line hFOB (all $p < 0.05$; Figures 1 E–G). Immunohistochemistry was conducted in osteosarcoma tissues, and the results also suggested high expression of CTNNBIP1 in osteosarcoma tissues (Figure 2).

Univariate and multivariate Cox regression analyses and nomogram construction

Both univariate and multivariate Cox regression analyses were applied to find independent prognostic predictors for forecasting OS from several clinical information points (primary tumor site, disease metastasis status at diagnosis, race, gender, and CTNNBIP1) in osteosarcoma. The results suggested that disease metastasis status at diagnosis (HR = 0.253) and CTNNBIP1 (HR = 0.213) were both independent predictors of OS in osteosarcoma (both $p < 0.05$; Figure 3 and Table I). With the assistance of independent prognostic predictors (disease metastasis status at diagnosis and the CTNNBIP1 gene), we also used a nomogram to intuitively display the 1-year, 3-year, and 5-year OS prognosis of osteosarcoma sufferers (Figure 4 A). Its performances were evaluated by 1-year, 3-year, and 5-year ROC curves, and the C-index, and their values were 0.941, 0.709, 0.704, and 0.723, respectively, with moderate diagnostic efficacy (Table II). One-year, 3-year, and 5-year calibration plots showed the good performance of this nomogram (Figures 4 B–D).

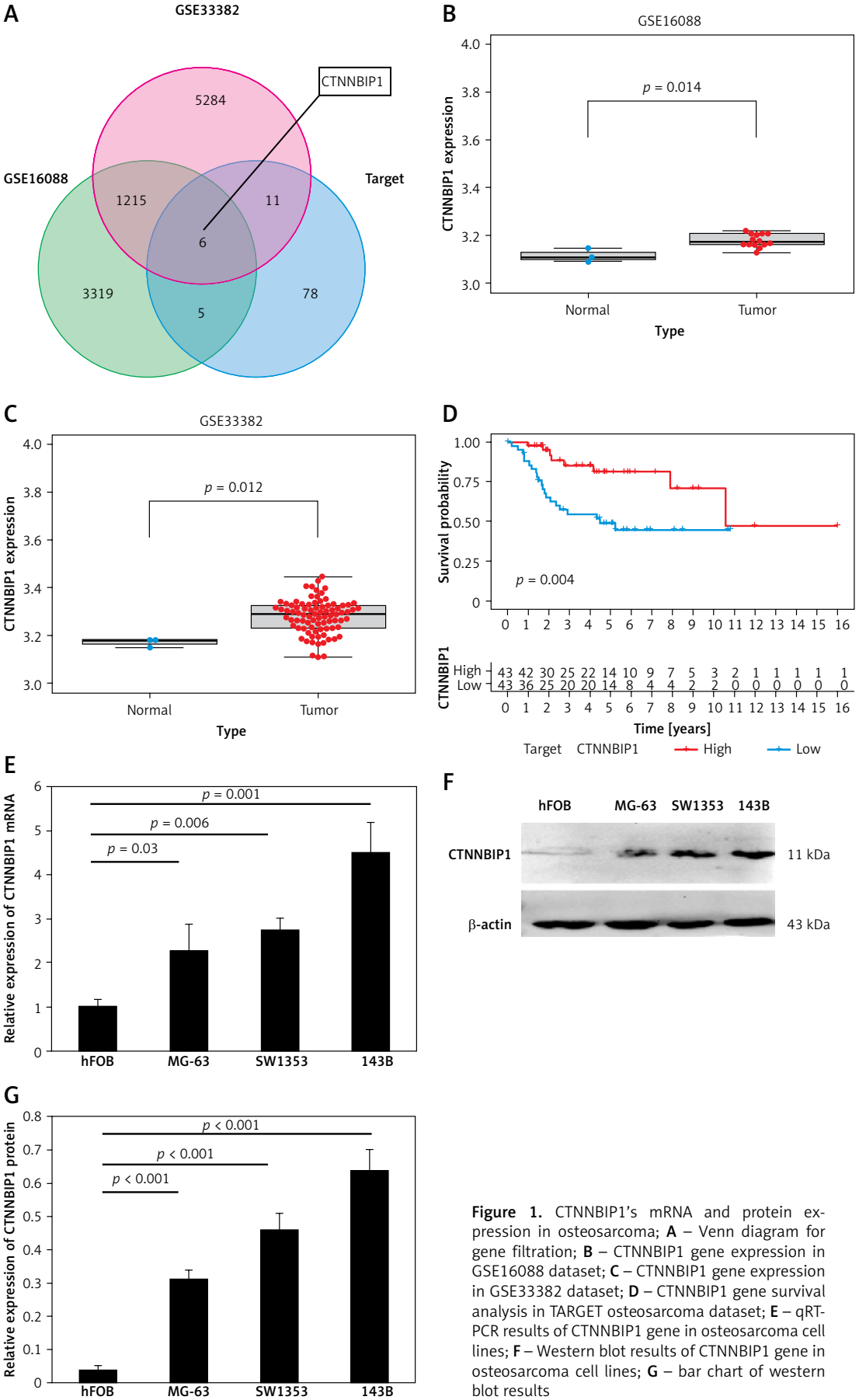
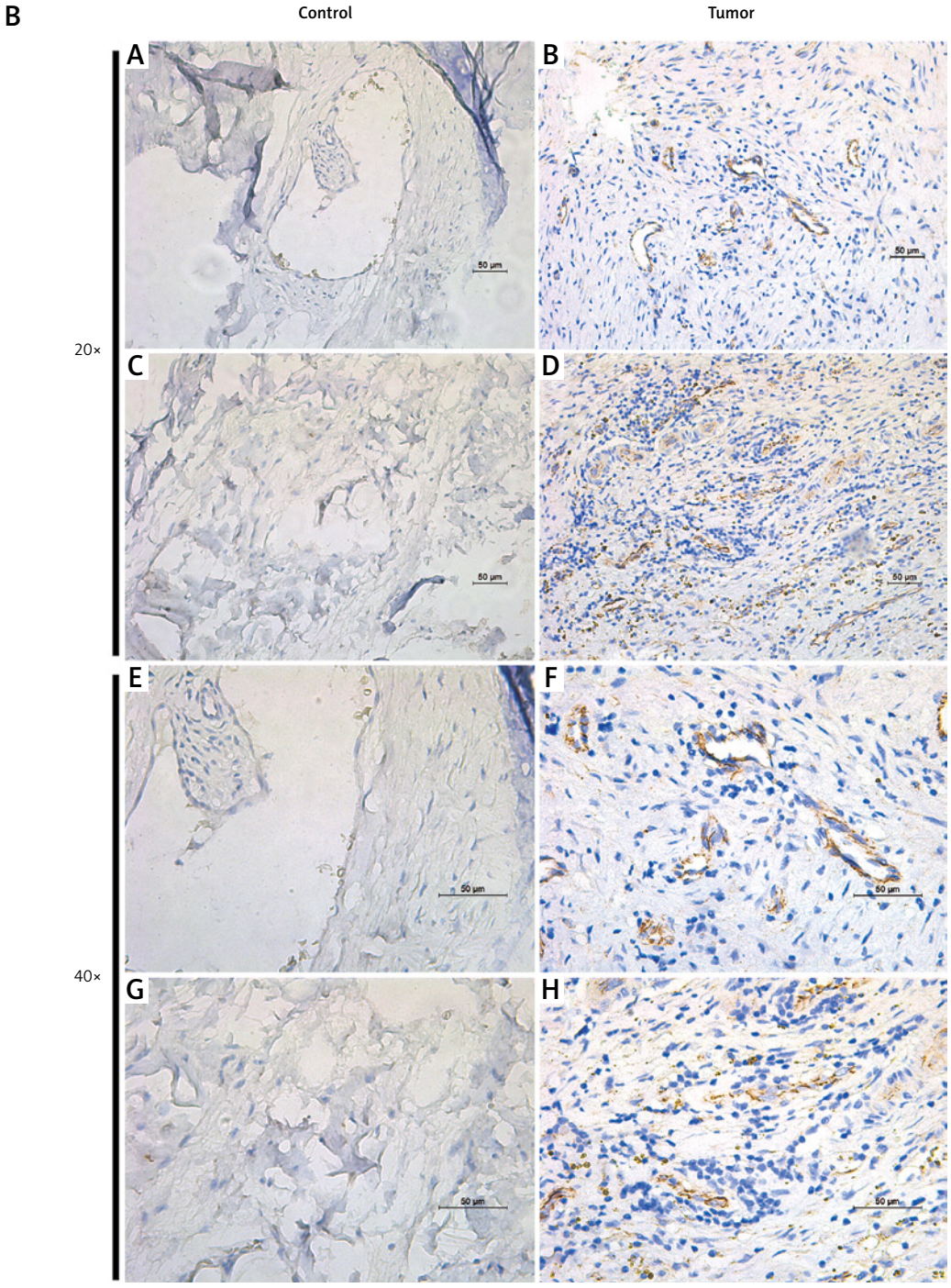


Figure 1. CTNNBIP1's mRNA and protein expression in osteosarcoma; **A** – Venn diagram for gene filtration; **B** – CTNNBIP1 gene expression in GSE16088 dataset; **C** – CTNNBIP1 gene expression in GSE33382 dataset; **D** – CTNNBIP1 gene survival analysis in TARGET osteosarcoma dataset; **E** – qRT-PCR results of CTNNBIP1 gene in osteosarcoma cell lines; **F** – Western blot results of CTNNBIP1 gene in osteosarcoma cell lines; **G** – bar chart of western blot results



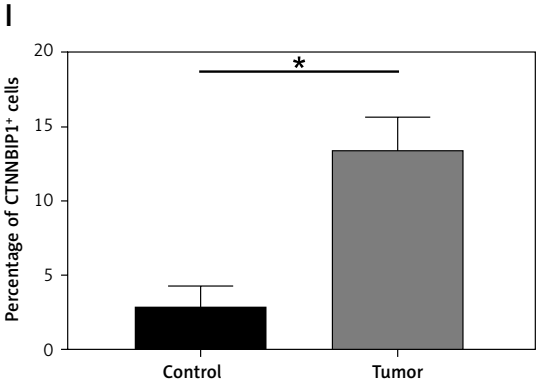


Figure 2. *In vivo* experiment of immunohistochemistry was conducted in (**A**, **C**) adjacent normal controls and (**B**, **D**) osteosarcoma tissues (magnification 20×); **E**, **G** – adjacent normal controls, **F**, **H** – osteosarcoma tissues (magnification 40×); **I** – quantification of immunohistochemistry staining

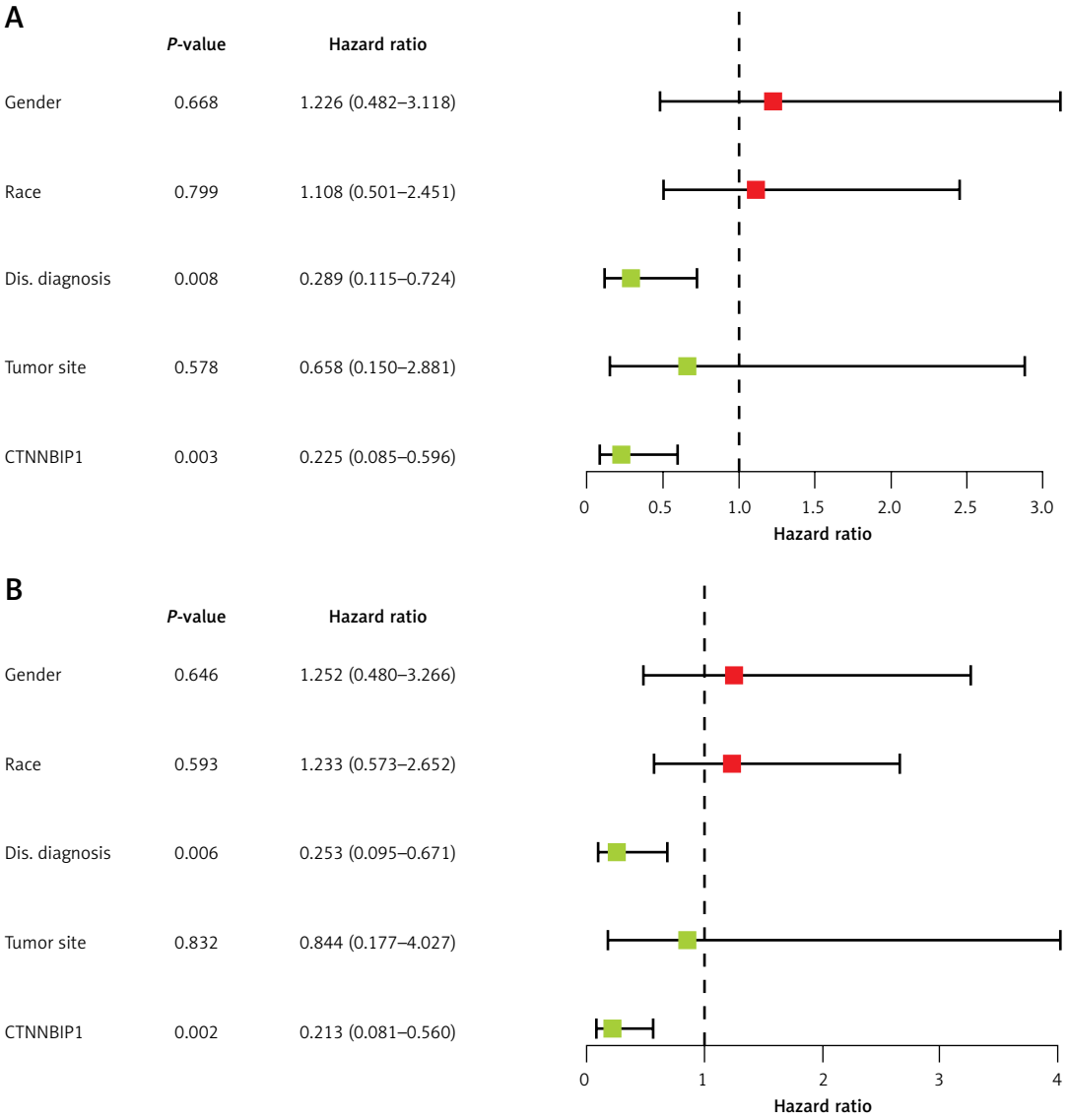


Figure 3. Univariate and multivariate Cox regression analyses; **A** – univariate Cox regression analyses; **B** – multivariate Cox regression analyses

Table I. Prediction of overall survival using univariate and multivariate analyses of CTNNBIP1 expression level and clinicopathological variables in target osteosarcoma dataset

Variables	Univariate analysis				Multivariate analysis			
	HR	HR 95L	HR 95H	*P*-value	HR	HR 95L	HR 95H	*P*-value
Gender	1.226386	0.482331	3.118234	0.66821	1.251809	0.479733	3.266451	0.646269
Race	1.108442	0.501196	2.451423	0.799314	1.232599	0.572894	2.651974	0.592676
Dis_diagnosis	0.288871	0.115323	0.723585	0.008036	0.252653	0.095099	0.671235	0.005787
Tumor_site	0.657825	0.150216	2.880754	0.578338	0.844375	0.177048	4.026977	0.831925
CTNNBIP1	0.225446	0.085229	0.596342	0.002686	0.212652	0.080801	0.559659	0.001715

Significantly CTNNBIP1-enriched pathways in osteosarcoma

We also applied gene set enrichment analysis (GSEA) to obtain significantly CTNNBIP1-enriched pathways in osteosarcoma, with the threshold of |normalized enrichment score (NES)| > 1.5 as well as p-values < 0.05. Our results demonstrated that CTNNBIP1 was significantly enriched in three pathways in osteosarcoma: the B cell receptor, nitrogen metabolism, and pancreatic cancer pathways (Figure 5 and Table III).

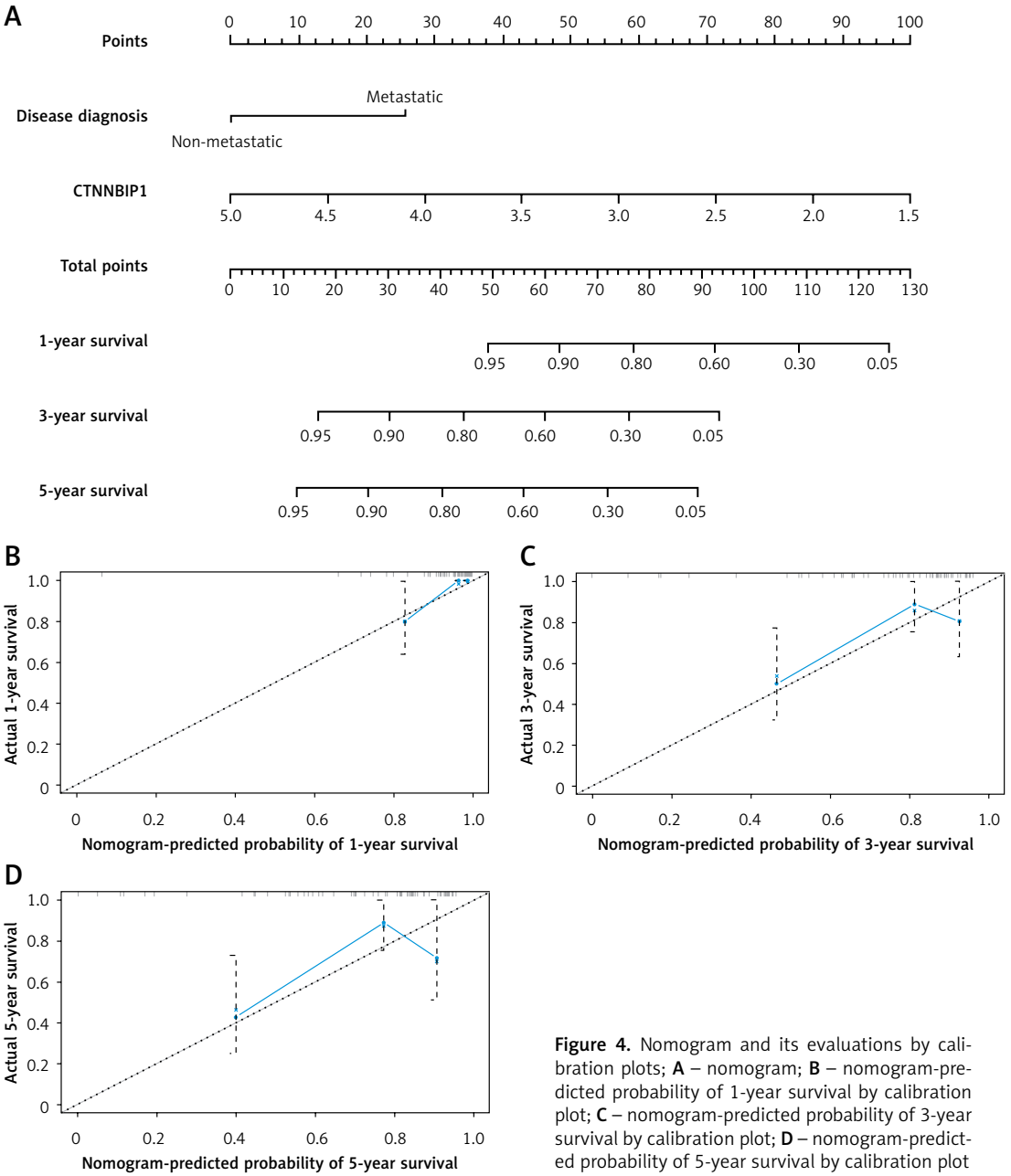


Figure 4. Nomogram and its evaluations by calibration plots; **A** – nomogram; **B** – nomogram-predicted probability of 1-year survival by calibration plot; **C** – nomogram-predicted probability of 3-year survival by calibration plot; **D** – nomogram-predicted probability of 5-year survival by calibration plot

Table II. C-index and 1-, 3-, 5-year AUCs of CTNNBIP1 based nomogram in target osteosarcoma dataset

	1-year	3-year	5-year	C-index
AUC	0.941	0.709	0.704	0.723

The immunologic roles of CTNNBIP1 in osteosarcoma

To reveal the immunologic roles of CTNNBIP1 in osteosarcoma, two aspects of immunity – tumor microenvironment and immune cell infiltrations – were further analyzed according to CTNNBIP1 gene expression. Immune cell infiltrations, including the calculations of the expression of 22 types of immune cells, were analyzed by the "CIBERSORT" algorithm and assessed for differences in high-CTNNBIP1 and low-CTNNBIP1 groups (Figures 6 A, B). Further correlation analysis showed that CTNNBIP1 was markedly associated with infiltration levels of naïve B cells and resting mast cells (Figures 6 C, D). Tumor microenvironment, including stromal, ESTIMATE, and immune scores, were analyzed by the "ESTIMATE" algorithm and assessed for differences in high-CTNNBIP1 and low-CTNNBIP1 groups (all $p < 0.05$; Figure 6 E). Further correlation analysis showed that CTNNBIP1 was markedly linked with stromal, ESTIMATE, and immune scores (all $p < 0.05$; Figures 6 F–H). We further explored the expression

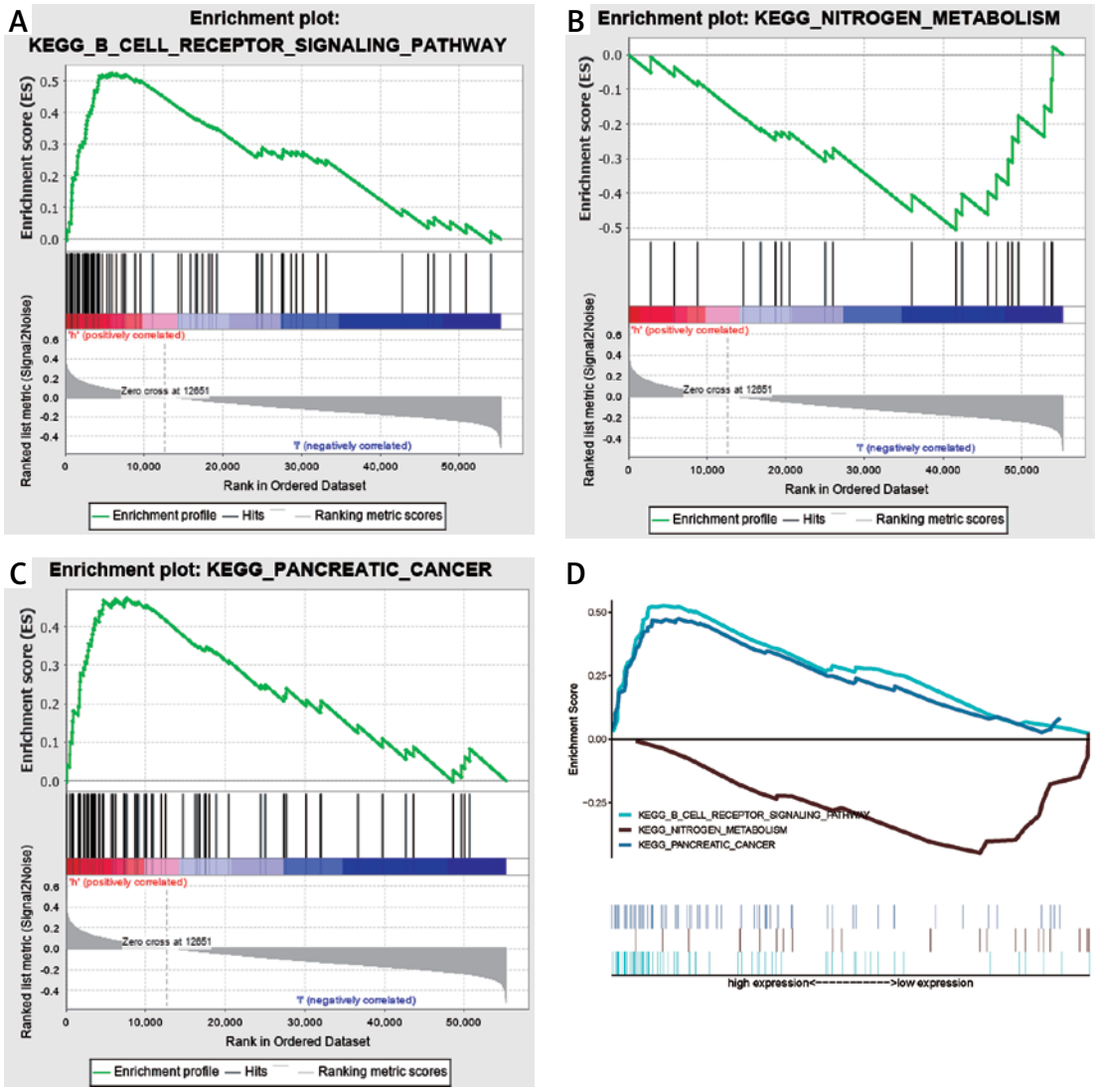


Figure 5. CTNNBIP1 significantly enriched pathways in osteosarcoma; **A** – B cell receptor pathway; **B** – nitrogen metabolism pathway; **C** – pancreatic cancer pathway; **D** – all of these three pathways

Table III. Gene set enrichment analysis of CTNNBIP1 mRNA expression in target osteosarcoma dataset

Gene set name	NES	NOM *P*-value	FDR q-value
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	1.801	0.015	0.217
KEGG_NITROGEN_METABOLISM	–1.568	0.034	1.000
KEGG_PANCREATIC_CANCER	1.866	0.011	0.327

NES – normalized enrichment score, NOM – nominal, FDR – false discovery rate.

of immune checkpoint genes, m6A genes, and B cell receptor pathway genes in high-CTNNBIP1 and low-CTNNBIP1 groups. Immune checkpoint genes such as CD44, TNFRSF9, TNFRSF8, HAVCR2, TNFRSF14, BTLA, m6A genes including HNRNPC and RBM15, and B cell receptor pathway genes including NFAT5, NFKB1, PTPN6, RAC2, NFKBIE, CHP2, JUN, PPP3R2, and LILRB3, were all differentially expressed in high-CTNNBIP1 and low-CTNNBIP1 groups (Figure 7).

Prediction of CTNNBIP1-related immune responses to immunotherapy and significantly CTNNBIP1-associated drugs

We used the TIDE dataset to predict the immune responses to immunotherapy in high-CTNNBIP1 and low-CTNNBIP1 groups. The results showed lower TIDE scores and T cell dysfunction scores in the high-CTNNBIP1 group, indicating that osteosarcoma patients with elevated CTNNBIP1 expression will have better immune re-

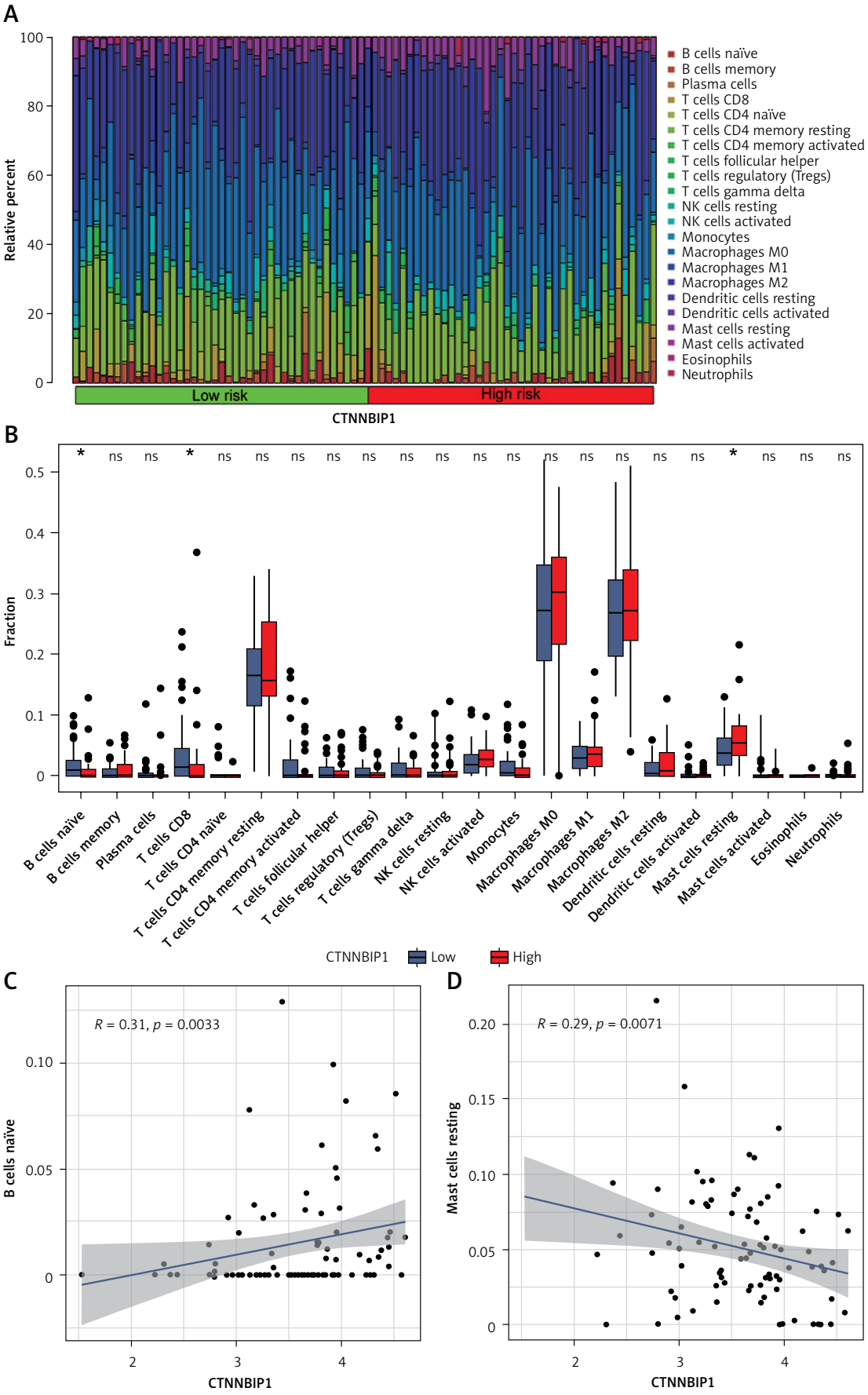


Figure 6. Correlations between CTNNBIP1 expression and immunity in osteosarcoma; **A** – proportions of tumor immune cells infiltration levels in tissues of TARGET osteosarcoma dataset; **B** – 22 kinds of immune cells in high-CTNNBIP1 and low-CTNNBIP1 subgroups; **C** – correlation analyses between CTNNBIP1 and naive B cell infiltration levels; **D** – correlation analyses between CTNNBIP1 and resting mast cell infiltration levels; *means $p < 0.05$; **means $p < 0.01$; ns means not significant

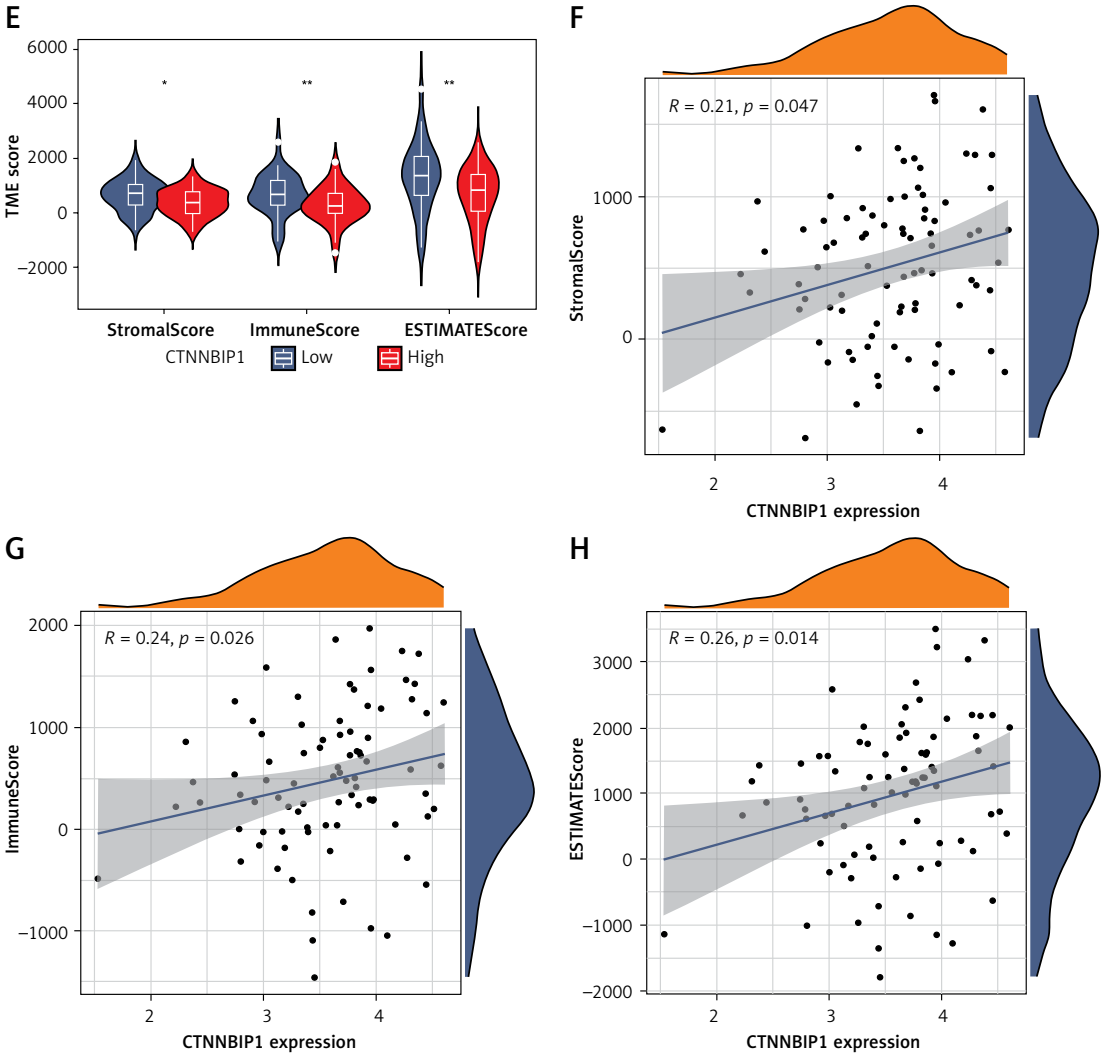


Figure 6. Cont. **E** – tumor microenvironment evaluations; **F** – correlation analyses between CTNNBIP1 and stromal score; **G** – correlation analyses between CTNNBIP1 and immune score; **H** – correlation analyses between CTNNBIP1 and ESTIMATE score; *means $p < 0.05$; **means $p < 0.01$; ns means not significant

sponses to immunotherapy (Figures 8 A, B). The Cellminer dataset was also applied to obtain significantly CTNNBIP1-associated drugs, with the threshold of |correlation coefficient| > 0.3 as well as *p*-values < 0.05. Our results showed that CTNNBIP1 was negatively correlated with AFP464 or aminoflavone drugs (Figures 8 C, D). These two negatively correlated drugs can be expected to have antitumor activity for future related drug development in osteosarcoma.

Discussion

Osteosarcoma is a common childhood and adolescent malignant bone tumor, characterized by frequent recurrence and distant metastasis. Despite the advances in therapeutic methods, the 5-year survival rates of osteosarcoma patients with metastasis or recurrence are still less than 20%. There is still an urgent need to elucidate the molecular mechanisms underlying osteosarcoma metastasis or recurrence and to identify novel therapeutic targets for future cancer precision medicine. The CTNNBIP1 gene has been reported to be involved in development, tumorigenesis, tissue differentiation, and other processes [8, 9]. However, the definite roles of CTNNBIP1 in osteosarcoma remained unclear. In this study, we investigated the prognostic roles of the CTNNBIP1 gene in osteosarcoma and its relationship with immunity using bioinformatics analysis and *in vitro*/*in vivo* experiments, providing reliable predictors for future cancer precision medicine.

Our findings suggested that the expression of CTNNBIP1 was higher in osteosarcoma than in normal tissues, and its high expression was correlated with increased OS. Further *in vitro* and *in vivo* experimental results of qRT-PCR, western blot, and immunohistochemistry remained consistent. Cox regression analyses, both univariate and multivariate, shed light on the metastasis

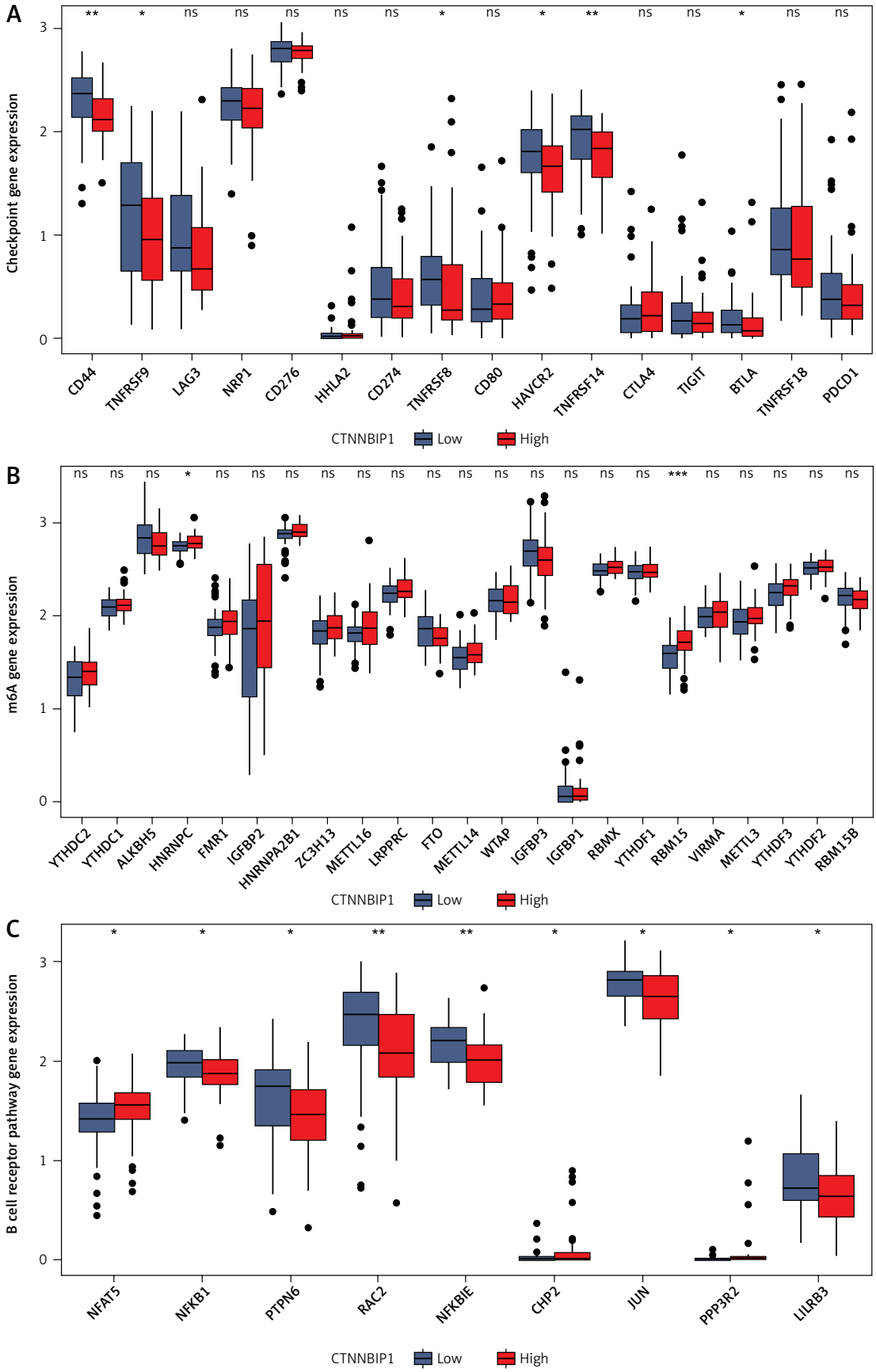


Figure 7. Expression of immune checkpoint genes (**A**), m6A genes (**B**) and B cell receptor pathway genes (**C**) in high-CTNNBIP1 and low-CTNNBIP1 groups; *means $p < 0.05$; **means $p < 0.01$; ***means $p < 0.001$; ns – means not significant

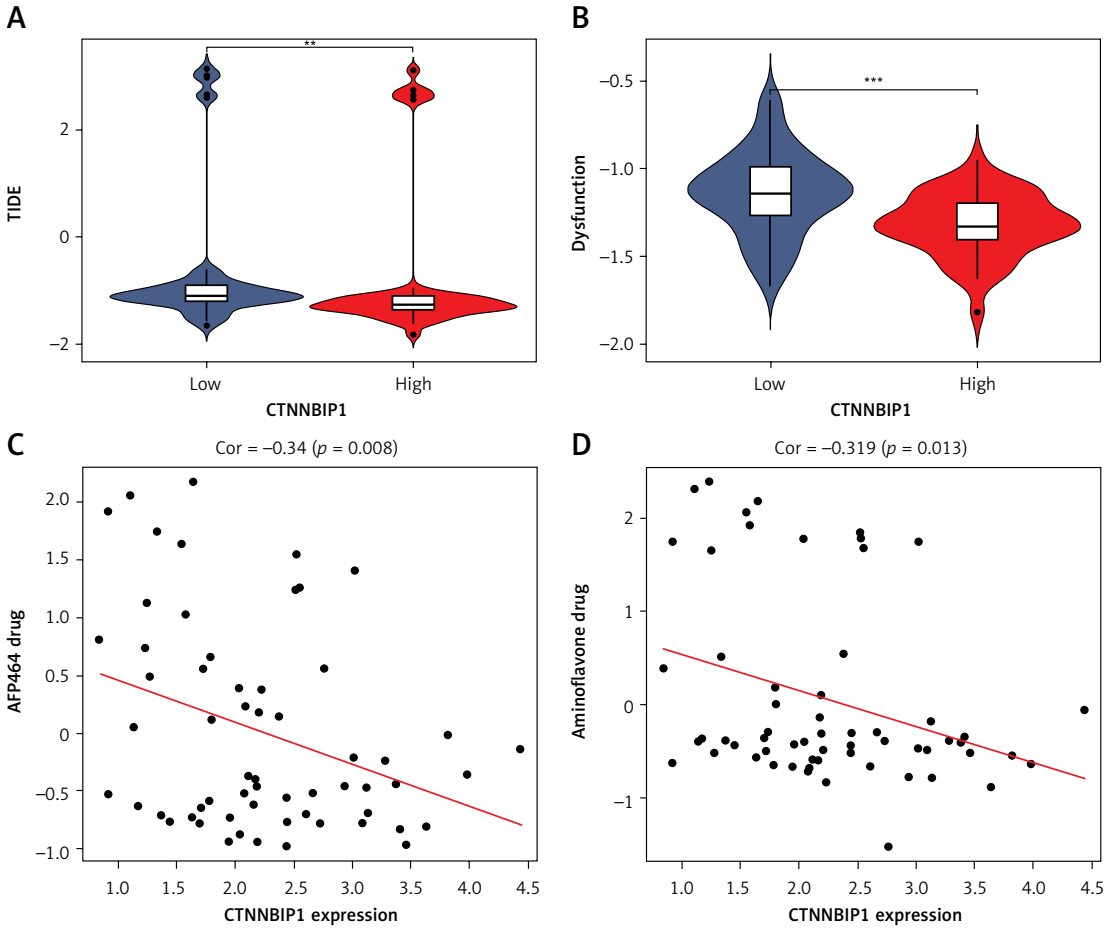


Figure 8. Prediction of CTNNBIP1-related immune responses to immunotherapy and significantly CTNNBIP1-associated drugs; **A** – TIDE scores in high-CTNNBIP1 and low-CTNNBIP1 groups; **B** – T cell dysfunction scores in high-CTNNBIP1 and low-CTNNBIP1 groups; **C** – associations between CTNNBIP1 and AFP464 by Cellminer dataset; **D** – associations between CTNNBIP1 and aminoflavone by Cellminer dataset; ** means $p < 0.01$; *** means $p < 0.001$

status of the disease at diagnosis, and CTNNBIP1 both had independent abilities to predict OS in osteosarcoma. GSEA results revealed that CTNNBIP1 was significantly enriched in three pathways, including the B cell receptor and nitrogen metabolism pathways, in osteosarcoma. As for the immunologic roles of CTNNBIP1 in osteosarcoma, CTNNBIP1 was shown to be markedly associated with infiltration levels of naïve B cells and resting mast cells, and tumor microenvironment. Furthermore, the TIDE algorithm indicated that osteosarcoma patients with elevated CTNNBIP1 expression will have a better immune response to immunotherapy. All in all, our findings suggested that CTNNBIP1 was significantly associated with patient survival and immune response, potentially serving as a novel biomarker for precision medicine in osteosarcoma.

To date, the role of the CTNNBIP1 gene in osteosarcoma has rarely been investigated, and only two articles are available. Rothzerg *et al.* identified 12 survival-based differentially expressed genes according to the TARGET osteosarcoma dataset for discovering novel therapeutic targets [15]. Yang *et al.* constructed and validated a 17-gene signature including CTNNBIP1 for forecasting OS in osteosarcoma patients [16]. To further mine significantly CTNNBIP1-enriched pathways in osteosarcoma, the GSEA results highlighted significant enrichment of CTNNBIP1 in the B cell receptor, nitrogen metabolism, and pancreatic cancer pathways, which had been previously linked to tumorigenesis in osteosarcoma. Notably, the B cell receptor pathway has been associated with prognosis in osteosarcoma. Guo *et al.* suggested that decreased STAT5A expression predicted poor prognosis and was markedly associated with the B cell receptor pathway in osteosarcoma [30]. Yin *et al.* developed an immune-based prognostic model for osteosarcoma, and GSEA also revealed its relationships with the B cell receptor pathway [31]. Yang *et al.* identified a high and a low immune cell infiltration subtype for predicting prognosis in osteosarcoma, and the B cell receptor pathway was markedly enriched in the high immune cell infiltration subtype [32]. The nitrogen me-

tabolism pathway has also been reported in cancer or osteosarcoma. In colon cancer, Chen *et al.* revealed its significant enrichment of the nitrogen metabolism pathway and mined MMP7 as a hub biomarker via bioinformatics analysis [33]. In osteosarcoma, Wu *et al.* developed a signature based on a hypoxic gene set for predicting OS, immunity, and chemosensitivity, and the nitrogen metabolism pathway was found to be activated in the high-risk group classified by the established signature [34]. The above-mentioned information showed that CTNNBIP1 markedly enriched pathways in osteosarcoma, providing a reference point for future CTNNBIP1-related *in vitro* and *in vivo* experiments in osteosarcoma.

Immunity has been reported to play vital roles in various cancers [35]. Sha *et al.* reported that cuproptosis-related genes were markedly linked to the tumor immunity of triple-negative breast cancer and could serve as a useful tool to predict these patients' prognosis [36]. Chen *et al.* showed that CLEC5A could serve as a predictor for forecasting cancer prognosis and immunity through pan-cancer analysis [37]. Wang *et al.* mined TCGA data and revealed that PANK1 was correlated with prognosis, immunity, and metabolism status in clear cell renal cell carcinoma [38]. In our study, immune cell infiltration results demonstrated that CTNNBIP1 was markedly associated with naïve B cell and resting mast cell infiltration levels. Cheng *et al.* first established a transcription factor-based model in ovarian cancer, and naive B cell infiltration levels were also markedly enriched in the high-risk group classified by the model [39]. As yet, no related articles have reported the associations between naïve B cell and mast cell resting infiltration levels and osteosarcoma. In our study, tumor microenvironment results showed that CTNNBIP1 was markedly associated with stromal, ESTIMATE, and immune scores. Huang *et al.* suggested the functions of cancer-associated fibroblasts in the tumor microenvironment of recurrent osteosarcoma using single-cell transcriptomics [40]. Ma *et al.* shed light on the relationships among prognosis, autophagy, and immunotherapy, as well as the tumor microenvironment, in osteosarcoma [41]. All in all, CTNNBIP1 was found to be markedly associated with immunity in osteosarcoma.

The TIDE algorithm has been widely used in various cancers to predict immune responses to immunotherapy [42, 43]. As for the potentially clinical application of CTNNBIP1 in osteosarcoma, our results from the TIDE algorithm suggest that elevated CTNNBIP1 expression correlates with improved immune response, particularly through reduced T cell dysfunction, supporting its potential role as a biomarker for immunotherapy in osteosarcoma patients. The Cellminer dataset has also been widely used in various cancers to predict gene-related drugs [44–46]. In the present study, the Cellminer dataset revealed that CTNNBIP1 was negatively correlated with AFP464 or aminoflavone drugs, suggesting that these two negatively correlated drugs will have antitumor activity for future related drug development in osteosarcoma.

This study identified CTNNBIP1 as a novel prognostic biomarker and potential target for immunotherapy in osteosarcoma. Further functional studies are needed to elucidate the precise mechanisms by which CTNNBIP1 regulates tumor progression and immune response. Our results are anticipated to provide a reference point for future CTNNBIP1-related experiments in osteosarcoma. More functional and mechanism experiments are still required to further elucidate the molecular mechanisms of CTNNBIP1's role in osteosarcoma.

In conclusion, the present study indicates, for the first time, that CTNNBIP1 may serve as a potential biomarker of prognosis and immunotherapy for osteosarcoma, with the assistance of *in vitro* and *in vivo* experiments. The GSEA results showed that CTNNBIP1 was significantly enriched in three pathways in osteosarcoma: B cell receptor, nitrogen metabolism, and pancreatic cancer pathways. The TIDE algorithm indicated that osteosarcoma patients with elevated CTNNBIP1 expression will have a better immune response to immunotherapy, and the Cellminer dataset revealed that CTNNBIP1 was negatively correlated with AFP464 or aminoflavone drugs, suggesting that these two negatively correlated drugs will have antitumor activity for future related drug development in osteosarcoma. Our results are anticipated to provide a reference point for future CTNNBIP1-related experiments, and more functional and mechanism experiments are still required to further reveal the molecular mechanisms of CTNNBIP1 involved in osteosarcoma.

Availability of data and material

The RNA-sequencing data and corresponding clinical information were downloaded from the Therapeutically Applicable Research to Generate Effective Treatments (TARGET; https://ocg.cancer.gov/programs/target) osteosarcoma tissue dataset. All data used to support the findings of this study are included within the article. Please contact the corresponding author for data requests.

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Ethical approval

This research was approved by the Institutional Research Ethics Committees of Affiliated Maternity and Child Healthcare Hospital of Nantong University (ethical code number: 2019-KO59). Osteosarcoma cell lines (MG-63, SW1353, 143B and hFOB) were purchased from Procell (Wuhan, China).

Conflict of interest

The authors declare no conflict of interest.

References

1. Philip T, Blay JY, Brunat-Mentigny M, et al. Osteosarcoma. Br J Cancer 2001; 84 (Suppl 2): 78-80.
2. Cortini M, Avnet S, Baldini N. Mesenchymal stroma: role in osteosarcoma progression. Cancer Letters 2017; 405: 90-9.
3. Isakoff MS, Bielack SS, Meltzer P, Gorlick R. Osteosarcoma: current treatment and a collaborative pathway to success. J Clin Oncol 2015; 33: 3029-35.
4. Meazza C, Scanagatta P. Metastatic osteosarcoma: a challenging multidisciplinary treatment. Expert Rev Anticancer Ther 2016; 16: 543-56.
5. Mialou V, Philip T, Kalifa C, et al. Metastatic osteosarcoma at diagnosis: prognostic factors and long-term outcome--the French pediatric experience. Cancer 2005; 104: 1100-9.
6. Qi W, Chen J, Cheng X, et al. Targeting the Wnt-regulatory protein CTNNBIP1 by microRNA-214 enhances the stemness and self-renewal of cancer stem-like cells in lung adenocarcinomas. Stem Cells 2015; 33: 3423-36.
7. Tago K, Nakamura T, Nishita M, et al. Inhibition of Wnt signaling by ICAT, a novel beta-catenin-interacting protein. Genes Develop 2000; 14: 1741-9.
8. Satoh K, Kasai M, Ishidao T, et al. Anteriorization of neural fate by inhibitor of beta-catenin and T cell factor (ICAT), a negative regulator of Wnt signaling. Proc Natl Acad Sci USA 2004; 101: 8017-21.
9. Fang XY, Pan HF, Leng RX, Ye DQ. Long noncoding RNAs: novel insights into gastric cancer. Cancer Letters 2015; 356: 357-66.
10. Ou MY, Xiao Q, Ju XC, et al. The CTNNBIP1-CLSTN1 fusion transcript regulates human neocortical development. Cell Rep 2021; 35: 109290.
11. Zhang Z, Jiang W, Hu M, Gao R, Zhou X. MiR-486-3p promotes osteogenic differentiation of BMSC by targeting CTNNBIP1 and activating the Wnt/β-catenin pathway. Biochem Biophys Res Commun 2021; 566: 59-66.
12. Chang JM, Tsai AC, Huang WR, Tseng RC. The alteration of CTNNBIP1 in lung cancer. Int J Mol Sci 2019; 20: 5684.
13. Bi W, Huang J, Nie C, et al. CircRNA circRNA_102171 promotes papillary thyroid cancer progression through modulating CTNNBIP1-dependent activation of β-catenin pathway. J Exp Clin Cancer Res 2018; 37: 275.
14. Kosari-Monfared M, Nikbakhsh N, Fattahi S, et al. CTNNBIP1 downregulation is associated with tumor grade and viral infections in gastric adenocarcinoma. J Cell Physiol 2019; 234: 2895-904.
15. Rothzerg E, Xu J, Wood D, Kõks S. 12 Survival-related differentially expressed genes based on the TARGET-osteosarcoma database. Exp Biol Med 2021; 246: 2072-81.
16. Yang J, Zhang A, Luo H, Ma C. Construction and validation of a novel gene signature for predicting the prognosis of osteosarcoma. Sci Rep 2022; 12: 1279.
17. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA 2005; 102: 15545-50.
18. Wang Y, Liu S, Chen Y, Zhu B, Xing Q. Survival prognosis, tumor immune landscape, and immune responses of PPP1R18 in kidney renal clear cell carcinoma and its potentially double mechanisms. World J Oncol 2022; 13: 27-37.
19. Maimaiti A, Tuersunniyazi A, Meng X, et al. N6-methyladenosine RNA methylation regulator-related alternative splicing gene signature as prognostic predictor and in immune microenvironment characterization of patients with low-grade glioma. Front Genet 2022; 13: 872186.
20. Meng S, Liu Y, Wang X, et al. The prognostic value and biological significance of gap junction beta protein 2 (GJB2 or Cx26) in cervical cancer. Front Oncol 2022; 12: 907960.
21. Gao Y, Li JY, Mao JY, Zhou JF, Jiang L, Li XP. Comprehensive analysis of CRIP1 expression in acute myeloid leukemia. Front Genet 2022; 13: 923568.
22. Zhang S, Zhang W, Zhang J. 8-Gene signature related to CD8(+) T cell infiltration by integrating single-cell and bulk RNA-sequencing in head and neck squamous cell carcinoma. Front Genet 2022; 13: 938611.
23. Lin Z, Wang R, Huang C, et al. Identification of an immune-related prognostic risk model in glioblastoma. Front Genet 2022; 13: 926122.
24. Xu JL. Wilms tumor 1-associated protein expression is linked to a T-cell-inflamed phenotype in pancreatic cancer. Dig Dis Sci 2022; 68: 831-40.
25. Reinhold WC, Sunshine M, Liu H, et al. CellMiner: a web-based suite of genomic and pharmacologic tools to explore transcript and drug patterns in the NCI-60 cell line set. Cancer Res 2012; 72: 3499-511.
26. Ma F, Li X, Fang H, Jin Y, Sun Q, Li X. Prognostic value of ANXA8 in gastric carcinoma. J Cancer 2020; 11: 3551-8.
27. Cai X, Qiu W, Qian M, et al. A candidate prognostic biomarker complement factor I promotes malignant progression in glioma. Front Cell Develop Biol 2020; 8: 615970.
28. Kui XY, Gao Y, Liu XS, et al. Comprehensive analysis of SLC17A9 and its prognostic value in hepatocellular carcinoma. Front Oncol 2022; 12: 809847.
29. Wei S, Yu Z, Shi R, et al. GPX4 suppresses ferroptosis to promote malignant progression of endometrial carcinoma via transcriptional activation by ELK1. BMC Cancer 2022; 22: 881.
30. Guo Z, Tang Y, Fu Y, Wang J. Decreased expression of STAT5A predicts poor prognosis in osteosarcoma. Pathol Res Pract 2019; 215: 519-24.
31. Yin CD, Hou YL, Liu XR, et al. Development of an immune-related prognostic index associated with osteosarcoma. Bioengineered 2021; 12: 172-82.
32. Yang M, Cao Y, Wang Z, Zhang T, Hua Y, Cai Z. Identification of two immune subtypes in osteosarcoma based on immune gene sets. Int Immunopharmacol 2021; 96: 107799.
33. Chen L, Ke X. MMP7 as a potential biomarker of colon cancer and its prognostic value by bioinformatics analysis. Medicine 2021; 100: e24953.
34. Wu F, Xu J, Jin M, et al. Development and verification of a hypoxic gene signature for predicting prognosis, immune microenvironment, and chemosensitivity for osteosarcoma. Front Mol Biosci 2021; 8: 705148.
35. Filaci G, Rizzi M, Setti M, et al. Non-antigen-specific CD8(+) T suppressor lymphocytes in diseases character-

ized by chronic immune responses and inflammation. Ann NY Acad Sci 2005; 1050: 115-23.
36. Sha S, Si L, Wu X, et al. Prognostic analysis of cuproptosis-related gene in triple-negative breast cancer. Front Immunol 2022; 13: 922780.
37. Chen R, Wu W, Chen SY, et al. A Pan-cancer analysis reveals CLEC5A as a biomarker for cancer immunity and prognosis. Front Immunol 2022; 13: 831542.
38. Wang B, Liu B, Luo Q, et al. PANK1 associates with cancer metabolism and immune infiltration in clear cell renal cell carcinoma: a retrospective prognostic study based on the TCGA database. Transl Cancer Res 2022; 11: 2321-37.
39. Cheng Q, Li L, Yu M. Construction and validation of a transcription factors-based prognostic signature for ovarian cancer. J Ovarian Res 2022; 15: 29.
40. Huang X, Wang L, Guo H, Zhang W, Shao Z. Single-cell transcriptomics reveals the regulative roles of cancer associated fibroblasts in tumor immune microenvironment of recurrent osteosarcoma. Theranostics 2022; 12: 5877-87.
41. Ma Y, Tong C, Xu M, He H, Chen C. Bioinformatics analysis reveals an association between autophagy, prognosis, tumor microenvironment, and immunotherapy in osteosarcoma. J Oncol 2022; 2022: 4220331.
42. Lei M, Luo C, Zhang J, Cao W, Ge J, Zhao M. A m(6) A methyltransferase-mediated immune signature determines prognosis, immune landscape and immunotherapy efficacy in patients with lung adenocarcinoma. Cell Oncol 2022; 45: 931-49.
43. Chen Y, Ji H, Liu S, Xing Q, Zhu B, Wang Y. Survival prognosis, tumor immune landscape, and immune responses of ADAMTS14 in clear cell renal cell carcinoma and its potential mechanisms. Front Immunol 2022; 13: 790608.
44. Ding X, Liu H, Yuan Y, Zhong Q, Zhong X. Roles of GFPT2 expression levels on the prognosis and tumor microenvironment of colon cancer. Front Oncol 2022; 12: 811559.
45. Huang R, Li L, Wang Z, Shen K. A systemic pan-cancer analysis of MPZL3 as a potential prognostic biomarker and its correlation with immune infiltration and drug sensitivity in breast cancer. Front Oncol 2022; 12: 901728.
46. Chen Y, Zhou M, Gu X, Wang L, Wang C. High expression of TACC3 is associated with the poor prognosis and immune infiltration in lung adenocarcinoma patients. Dis Markers 2022; 2022: 8789515.