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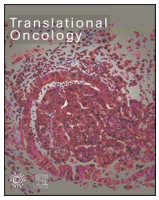

Original Research

Low NT5DC2 expression predicts favorable prognosis and suppresses soft tissue sarcoma progression via ECM-receptor interaction pathway

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ABSTRACT

Background: Soft tissue sarcoma, a malignant tumor arising from mesenchymal tissues with poor prognosis. 5′-Nucleotidase Domain Containing 2 (NT5DC2) is a novel oncogene, and the precise involvement of NT5DC2 in soft tissue sarcoma were still undefined. Hence, our study aims to investigate NT5DC2 functions in soft tissue sarcoma progression.

Methods: The tumor immune single-cell hub 2 (TISCH2) website, The Cancer Genome Atlas (TCGA) pan-cancer or sarcoma and Gene Expression Omnibus (GEO, GSE21122) databases were applied to visualize the NT5DC2 status in the sarcoma databases. The NT5DC2 protein expression in sarcoma tissues in our hospital was detected by using immunohistochemistry (IHC) and analyzed the associations between NT5DC2 expression and clinicopathological parameters. Real-time quantitative polymerase chain reaction (RT-qPCR), colony formation, 5-ethynyl-2′-deoxyuridine (EdU) assay, wound healing, transwell, flow cytometry and xenograft model were used to elucidate the effects of NT5DC2 downregulated by lentivirus in sarcoma cell.

Results: The TISCH2 website detection found that NT5DC2 expression is enriched in malignant cells in sarcoma single-cell database. Furthermore, the TCGA-sarcoma database indicated that NT5DC2 expression correlates with metastasis, positive margin status, prognosis, and diagnostic value. Additionally, IHC staining showed that 40 % of soft tissue sarcoma patients present high expression of NT5DC2, and NT5DC2 upregulation is closely associated with poor prognosis. Functional verification analysis further revealed that downregulating NT5DC2 expression can suppress sarcoma progression through the ECM-receptor interaction pathway.

Conclusion: Low expression of NT5DC2 predicts a favorable prognosis in soft tissue sarcoma, and downregulated NT5DC2 expression can suppress sarcoma cell progression through the ECM-receptor interaction pathway.

Introduction

Soft tissue sarcoma is a heterogeneous series of tumors derived from mesenchymal tissue, may lead to severe disability and death [1,2]. According to epidemiological data published by the American Cancer Society, 13,460 new cases are expected in 2021, of which 5350 patients will die from soft tissue sarcoma [3]. Common soft tissue sarcomas include malignant fibrous histiocytoma, synovial sarcoma, liposarcoma, rhabdomyosarcoma, etc. [4,5]. Soft tissue sarcoma has a poor prognosis, with an overall 5-year survival rate of about 50 % to 60 % [6,7]. Although the morphology of soft tissue sarcomas is characterized by spindle cells and signs of partial mesenchymal differentiation, the actual classification of soft tissue sarcomas is extremely complex, which often leads to delays in diagnosis and clinical strategy development [8]. At present, the main treatment of soft tissue sarcoma is surgical resection combined with preoperative or postoperative chemoradiotherapy [9]. In recent years, systemic therapy which refers to system anti-tumor therapy, containing chemotherapy, radiotherapy, targeted therapy and immunotherapy, is playing an increasingly important roles in the treatment of soft tissue sarcomas [10–13]. However, the improvement of prognosis in sarcoma patients is still limited.

5′-Nucleotidase Domain Containing 2 (NT5DC2) is a member of the NT5DC family with a halo-acid dehalogenase motif localized in the N-terminus of these proteins [14]. Recently, studies reported that NT5DC2 protein has emerged as a potential contributor to tumorigenesis. Yu et al. [15] found that high expression of NT5DC2 indicate a poor prognosis in

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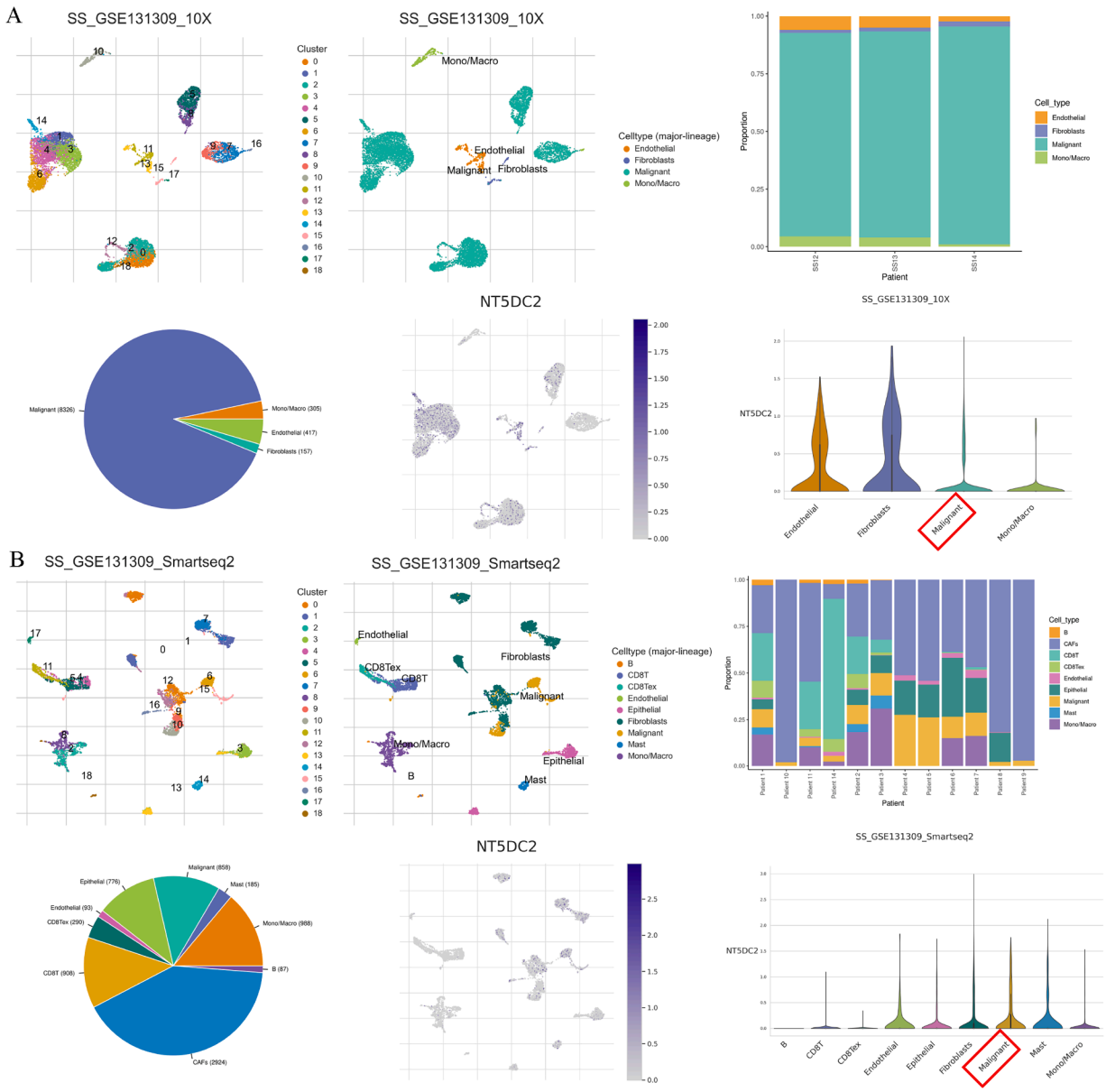

Fig. 1. The expression status of NT5DC2 in sarcoma single-cell database. (A) The expression of NT5DC2 is enriched in malignant cell type in SS_GSE131309_10X database; (B) NT5DC2 expression is enriched in malignant cell type in SS_GSE131309_Smartseq2 database.

pancreatic adenocarcinoma patients. Similarly, Schulze et al. [16] demonstrated that NT5DC2 upregulation is a negative prognostic marker in pulmonary adenocarcinoma. While, the functions of NT5DC2 expression in soft tissue sarcoma progression were explored inadequately. Hence, our study aimed to address this gap by exploring NT5DC2 status in soft tissue sarcoma.

Materials and methods

Data download and pre-process

Sarcoma_GSE131309_10X and Sarcoma_GSE131309_Smartseq2 datasets were obtained from tumor immune single-cell hub 2 (TISCH2) website (http://tisch.compgenomics.org/) [17]. GSE21122 expression profile was acquired from the Gene Expression Omnibus dataset (https://www.ncbi.nlm.nih.gov/geo/), and TCGA-Sarcoma from the TCGA dataset (https://portal.gdc.cancer.gov/).

The expression levels of NT5DC2 in sarcoma and its clinical significance

The status of NT5DC2 in the sarcoma-related single-cell database was analyzed on the TISCH2 website. The expression of NT5DC2 in TCGA-Sarcoma and GSE21122, and its associations with clinicopathological parameters, and survival status were detected by R software. Moreover, we explore the Gene Ontology (GO): Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment pathway in NT5DC2-related genes in the TCGA-Sarcoma database.

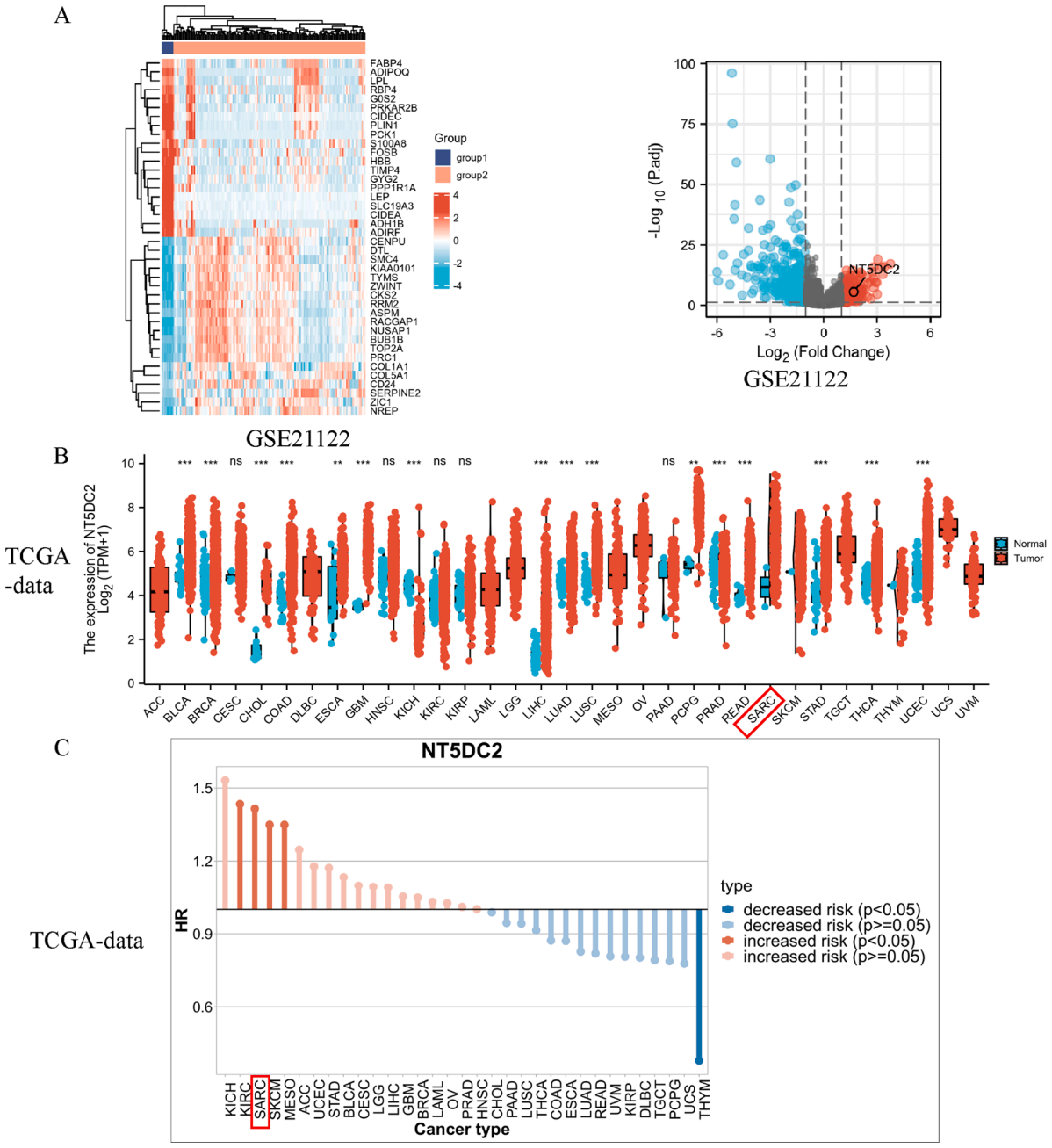

Fig. 2. The expression of NT5DC2 in GSE21122 and TCGA-pan-cancer database. (A) NT5DC2 is overexpressed in GSE21122 database; (B) NT5DC2 is upregulated in many types of tumors; (C) The expression of NT5DC2 predicts an increased risk in sarcoma group in TGCA data.

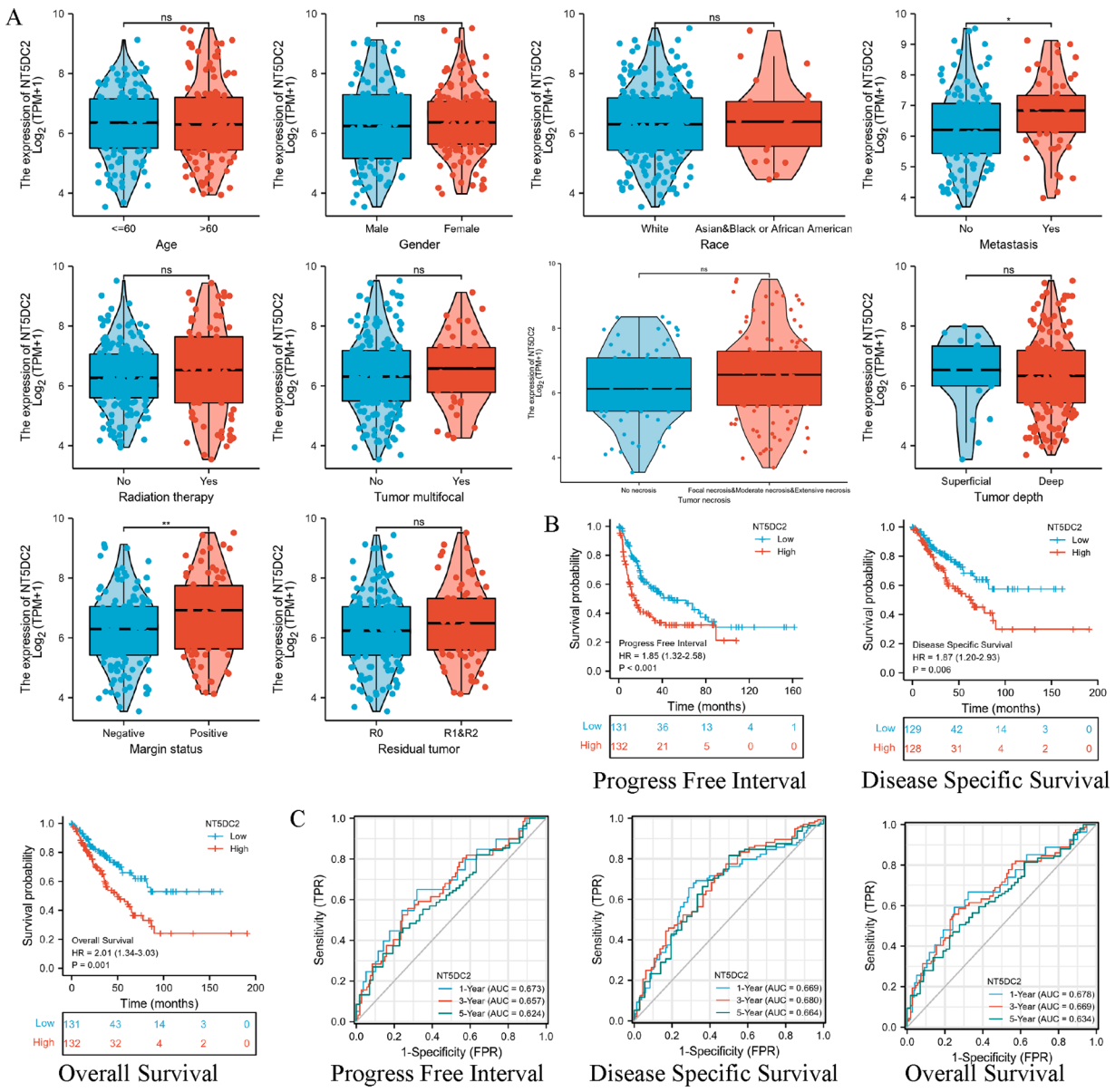

Fig. 3. The associations between NT5DC2 and clinicopathological parameters, and sarcoma patient's survival. (A) High expression of NT5DC2 is closely related to metastasis and margin status in TCGA-sarcoma database; (B) High expression of NT5DC2 associated with poor PFI, DSS and OS in TCGA-sarcoma group; (C) The diagnostic values of NT5DC2 expression in PFI, DSS and OS in TGCA-sarcoma.

Patients

Forty soft tissue sarcoma patients who underwent surgical resection treatment in the Department of Orthopedics, Shanghai Tenth People's Hospital, School of Medicine, Tongji University (Shanghai, China) between 1 January 2014 and 31 December 2021 were selected. The clinical information of these patients was acquired from the records in our hospital which including gender, age, tumor size, tumor location, metastasis, tumor depth, recurrence and survival status. The progression-free survival (PFS) time was defined as the date of diagnosis to the first tumor progression, and overall survival (OS) time was defined as the date of diagnosis to the death date.

Immunohistochemistry (IHC) staining

IHC assay was used to detect the expression of NT5DC2 protein in soft tissue sarcoma. Firstly, the sections were deparaffined, hydrated, and blocked endogenous peroxidase activity with 3 % hydrogen peroxide. Then, citrate buffer (pH 6.0) was used for antigen retrieval, and incubated with rabbit polyclonal anti-NT5DC2 antibody (bs-19491R, 1:200, Bioss Antibodies, China) in 4 °C overnight. On the next day, the sections were incubated with poly peroxidase-anti-rabbit IgG at room temperature for 30 mins and diaminobenzidine at room temperature for 5 mins. Two independent pathologists evaluated the staining results.

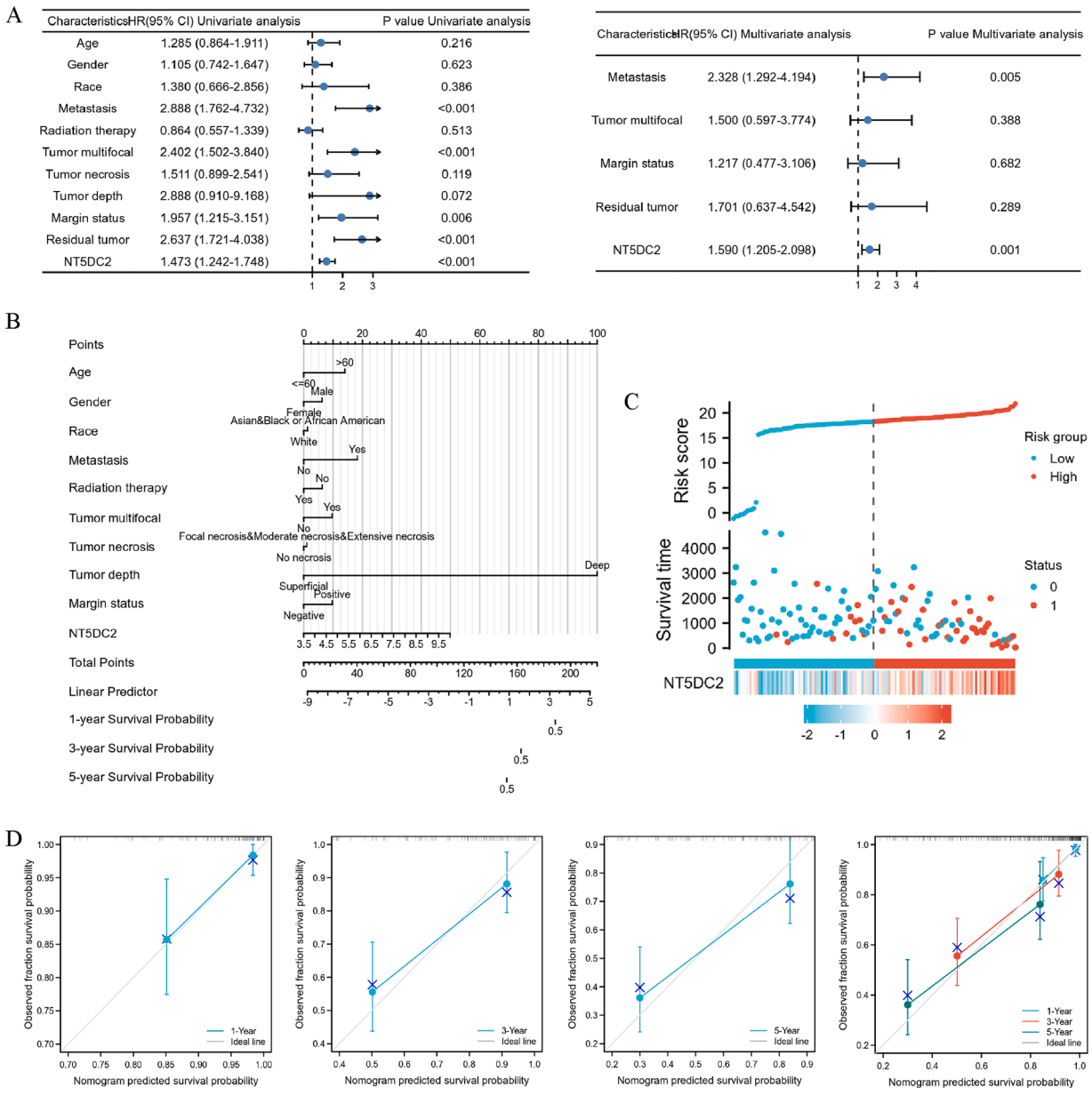

Fig. 4. The prognostic value of NT5DC2 in TCGA-sarcoma. (A) The univariate and multivariate analysis showed that NT5DC2 can be an independent prognostic biomarker in soft tissue sarcoma; (B) The nomogram analysis of clinicopathological parameters and NT5DC2 in TCGA-sarcoma database; (C) The survival status in low-risk and high-risk of NT5DC2 in TCGA-sarcoma; (D) The calibration curve of 1, 3, 5 years of NT5DC2 in TCGA-sarcoma.

Cell culture

HT1080 and SW872 cells were purchased from the National Collection of Authenticated Cell Cultures (Shanghai, China). Cells were cultured in high glucose of Dulbecco' s modified Eagle' s medium (Viva Cell Bioscience, Shanghai, China) with 10 % fetal bovine serum (FBS, Viva Cell Bioscience, Shanghai, China) and 1 % penicillin/streptomycin (Viva Cell Bioscience, Shanghai, China).

Lentivirus transfection

The sh-NC and sh-NT5DC2 lentivirus were obtained from Gemma Gene (Suzhou, China). Cells ($5 \times 10^{\wedge 4}$ per well) was seeded in six-well plates and cultured overnight. Then, a fresh medium replaced the medium, and the lentivirus was used to transfect cells according to the manufacturer's introductions.

5-ethynyl-2′ -deoxyuridine (EdU) assay

Sh-NC ($5 \times 10^{\wedge 4}$ cell) and sh-NT5DC2 ($5 \times 10^{\wedge 4}$ cell) cells were planted in 24 well plates for overnight incubation. Then, a 500μl medium with a 10μM concentration of EdU solution was used to incubate cells for 2 h at 37 °C. After that, cells were fixed by using 4 % paraformaldehyde for 15 mins, and Apollo dyeing reaction solution was

Table 1
The clinicopathological characteristics of soft tissue sarcoma patients.

Clinicopathological Data	n%
Gender	
Male	17 (42.5)
Female	23 (57.5)
Age (years)	
>60	16 (40)
≤60	24 (60)
Tumor size (cm)	
>8	19 (47.5)
≤8	21 (52.5)
Tumor location	
Thigh	16 (40)
others	24 (60)
Metastasis	
Yes	22 (55)
No	18 (45)
Tumor depth	
Deep	30 (75)
Superficial	10 (25)
Recurrence	
Yes	17 (42.5)
No	23 (57.5)
Survival outcome	
Disease-free	18 (45)
Alive with disease	7 (17.5)
Succumbed to disease	15 (37.5)

applied to incubate cells for 30 mins without light. The nuclei were stained using Hoechst and assessed under the microscope.

Colony formation

A density of 600 cells in Sh-NC and sh-NT5DC2 groups were seeded in 6 well plates and incubated for six days with a 5 ml complete medium. Then, cells were fixed with 4 % paraformaldehyde for 20 mins and stained with 0.1 % crystal violet (Solarbio, Beijing, China) for 20 mins. A colony of more than 50 cells were counted.

Wound healing

Sh-NC ($5 \times 10^{\wedge 5}$ cell) and sh-NT5DC2 ($5 \times 10^{\wedge 5}$ cell) cells were seeded in 6 well plates and cultured overnight. A sterile pipette tip (200 μl) was used to scrape cells, and the wound healing conditions were assessed by using a microscope at 0 h and 36 h.

Transwell assay

Migration and invasion abilities were assessed by using a transwell assay. Chamber (8 μm pore size, Corning, USA) was applied to conduct a transwell assay with the absence of Matrigel (BD Biosciences, USA) for migration and the presence of Matrigel (BD Biosciences, USA) for invasion in upper chamber. Sh-NC ($5 \times 10^{\wedge 4}$ cell/ per well) and sh-NT5DC2 ($5 \times 10^{\wedge 4}$ cell/ per well) combined with 200μl medium without FBS were seeded in the upper chamber, and 600μl complete medium in the lower chamber. Following 24 h of incubation, 4 % paraformaldehyde was applied to fix the migrated and invaded cell for 20 mins and stained by 0.1 % crystal violet (Solarbio, Beijing, China). The number of migrated and invaded cells were quantified under a microscope.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from sh-NC and sh-NT5DC2 cells by using an RNA-Quick Purification Kit (ShareBio, Shanghai). ABScript Neo RT Master Mix for qPCR with gDNA Remover (ABclonal, Wuhan) was applied to revere RNA to cDNA, and Taq SYBR Green qPCR Premix Kit (iScience, China) was used to amplify the cDNA on QuantStudio 1 (Thermo Fisher Scientific Inc, USA). The primers were purchased from Sangon Biotech (Shanghai, China), and the sequence are: NT5DC2, Forward: TGCCAGACGAGGAGGTGATTGAG, Reverse: CGGTAGCGAGAAGATGTCCATGAAC; COL1A1, Forward: GGCAAAGAAGGCGGCAAAGG, Reverse: GGAGCACCAGCAGGACCATC; COL1A2, Forward: CGTGGCAGTGATGGAAGTGTG, Reverse: ACCAGCAGGACCAGCGTTAC; COL6A1, Forward: AGCACCTGGGCGTCAAAGTC, Reverse: TGTGGTCCGTGGCGATGATG; COL6A2, Forward: GCTTCAAGGAGGCTGTCAAGAAC, Reverse: TTGATGAGGCGGTCGTAGGC; COL6A3, Forward: GTTCCTGGTCCTCATCTCGTCTG, Reverse: CTCCTCCTGGTCTGCGTTCC; IBSP, Forward: AGAGGAGGAGGAAGAAGAGGAGAC, Reverse: GCCCAGTGTTGTAGCAGAAAGTG; SDC1, Forward: CAAGGAGGGAGAGGCTGTAGTC, Reverse: TGGTGGCTGTGGTCGTTGAG; ITGA10, Forward: CTGCTCTGACTGACATTGTGGATG, Reverse: CTTTAGCCGATGAGTGGAGAAACC; and the housekeeping gene GAPDH, Forward: TGACATCAAGAAGGTGGTGAAGCAG, Reverse: GTGTCGCTGTTGAAGTCAGAGGAG.

Xenograft tumor model

Animal experiments were permitted by Shanghai Tenth People's Hospital, School of Medicine, Tongji University. The female nude mice were purchased from Shanghai SLAC Laboratory Animal Company. Sh-NC ($2 \times 10^{\wedge 6}$ cell) and sh-NT5DC2 ($2 \times 10^{\wedge 6}$ cell) HT1080 cells were injected into the left axilla of nude mice. Mice were euthanized when the tumor volume approached to $2000mm^3$.

Statistical analysis

R software (4.1.2) was used to pre-process the data and analyze the status of NT5DC2 in the TGCA-Sarcoma database. GraphPad Prism 8.3.0 (USA) was performed to explore the associations between NT5DC2 expression and clinicopathological parameters in soft tissue sarcoma. The Kaplan-Meier survival plots were employed to investigate the associations between NT5DC2 status and survival outcome. Cox hazard regression analysis was performed to evaluate the univariate and multivariate analysis of NT5DC2 expression in sarcoma patients' survival. Student's *t*-test and mean ± SD were used to analyze the difference between the two groups. A p-value less than 0.05 indicated statistical significance.

Results

NT5DC2 expressions in sarcoma-related single-cell, GEO and TCGA database

The expression status of NT5DC2 in a sarcoma-related single-cell database was explored on the TISCH2 website. The results revealed a conspicuous enrichment of NT5DC2 expression in malignant cells in SS_GSE131309_10X and SS_GSE131309_Smartseq2 database (Fig. 1). Moreover, the analysis of sarcoma-related GSE21122 database found a significant upregulation of NT5DC2 gene in sarcoma specimens (Fig. 2A). Additionally, we analyzed the expression NT5DC2 in the TCGA pan-cancer database and showed that NT5DC2 is overexpressed in

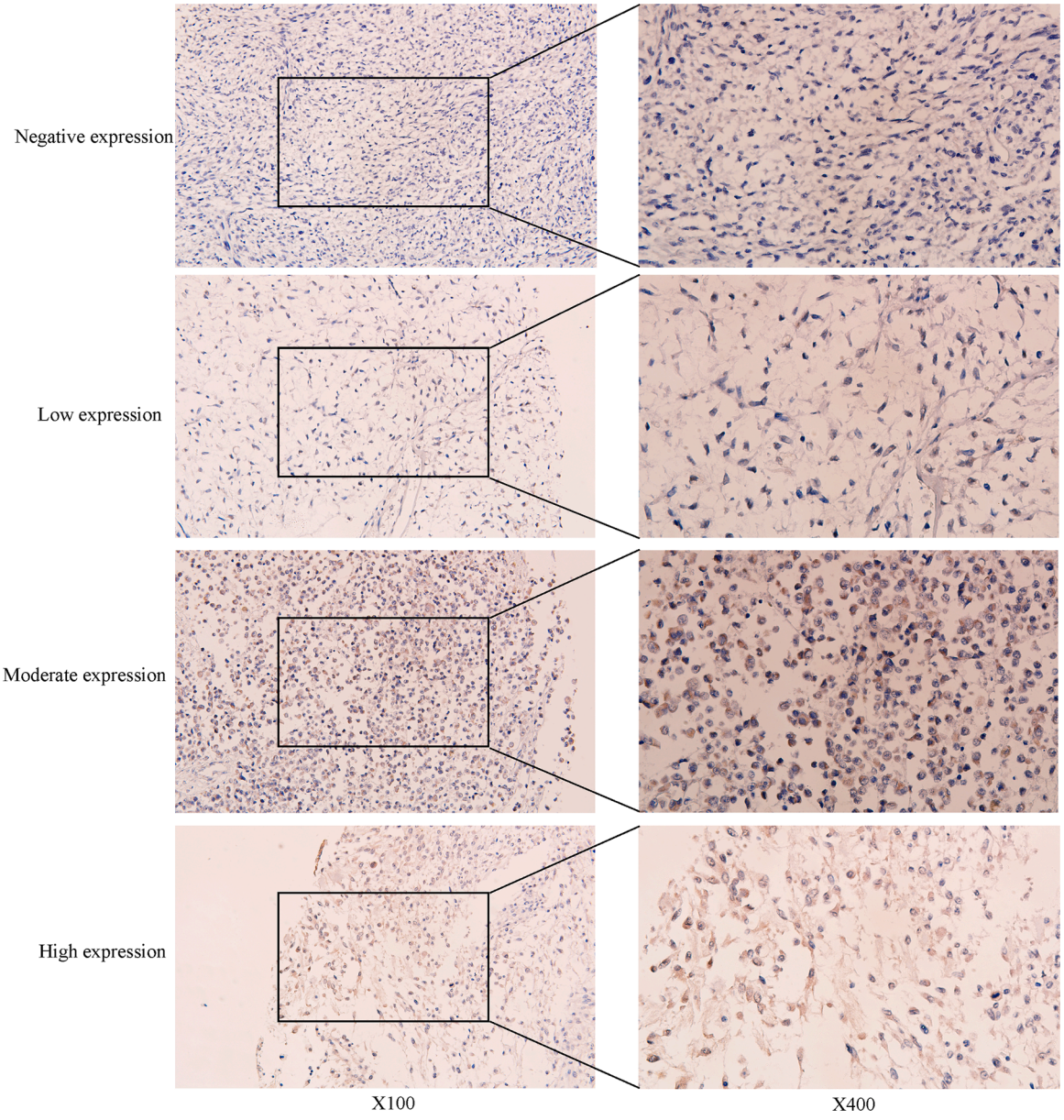

Fig. 5. Different expression levels of NT5DC2 protein in soft tissue sarcoma.

most types of cancer and can significantly increase sarcoma group risk (Fig. 2B, C).

The associations between NT5DC2 expression and clinicopathological parameters, survival status and diagnostic values from TCGA-sarcoma database

The analysis of NT5DC2 status within the TCGA-sarcoma database revealed that NT5DC2 upregulations is closely related to metastasis and margin positive status (Fig. 3A). Kaplan-Meier survival plots analysis found that high expression of NT5DC2 predicts a poor progress-free interval (PFI), disease-specific survival (DSS), and overall survival (OS) in sarcoma from the TCGA database (Fig. 3B). The Receiver Operating Characteristic Curve of PFI, DSS, and OS in 1, 3, and 5 years in the TCGA-sarcoma database indicates a relatively notable diagnostic accuracy (Fig. 3C).

The prognostic values of NT5DC2 in TCGA-sarcoma database

The prognostic values of NT5DC2 expression in sarcoma patients were evaluated by Cox regression analysis. The univariate analysis demonstrated that metastasis, tumor multifocal, margin status, residual tumor and NT5DC2 expression are associated with PFS and OS. Subsequently, the multivariate analysis identified metastasis and NT5DC2 expression as potentially independent prognostic factors in soft tissue sarcoma (Fig. 4A), and high expression of NT5DC2 presents a shorter

Table 2
The associations between NT5DC2 expression and clinicopathological characteristics of soft tissue sarcoma patients.

Clinicopathological Data	Case Number	High (n = 16)	Low (n = 24)	P value
Gender				
Male	17	7	10	0.896
Female	23	9	14	
Age (years)				
>60	16	6	10	0.792
≤60	24	10	14	
Tumor size (cm)				
>8	19	10	9	0.121
≤8	21	6	15	
Tumor location				
Thigh	16	8	8	0.292
others	24	8	16	
Metastasis				
With	22	11	11	0.154
Without	18	5	13	
Tumor depth				
Deep	30	10	20	0.136
Superficial	10	6	4	
Recurrence				
Yes	17	8	9	0.433
No	23	8	15	

survival time and higher risk (Fig. 4B, C). Furthermore, the nomogram validation confirmed that NT5DC2 could be a robust well-diagnostic biomarker in sarcoma (Fig. 4D).

NT5DC2 protein expression in soft tissue sarcoma and its associations with clinicopathological parameters in our hospital

The clinicopathological characteristics of the cohort are summarized in Table 1. IHC staining was employed to evaluate NT5DC2 protein expression level in 40 sarcoma tissues (fibrosarcoma, leiomyosarcoma, liposarcoma, Ewing's sarcoma, undifferentiated pleomorphic sarcoma, etc.). The staining of NT5DC2 protein showed that NT5DC2 is overexpressed in 40 % (16/40) of the sarcoma tissues, as shown in Fig. 5. Subsequent analysis of the relationships between NT5DC2 expression and clinicopathological parameters revealed that high expression of NT5DC2 is not associated with any other clinicopathological data (Table 2).

The status of NT5DC2 in soft tissue sarcoma patients' survival

Kaplan-Meier survival plots were applied to investigate the relationships between NT5DC2 expression and PFS and OS times in soft tissue sarcoma patients which found that with metastasis and high expression of NT5DC2 present a poor PFS and OS times in soft tissue sarcoma, as shown in Fig. 6A. Moreover, the univariate analysis found that metastasis (PFS, $P < 0.001$; OS, $P = 0.001$) and NT5DC2 (PFS, $P = 0.013$; OS, $P = 0.008$) with PFS and OS in soft tissue sarcoma (Table 3). The multivariate analysis further corroborated that NT5DC2 can be an independent prognostic biomarker in poor PFS and OS outcomes in soft tissue sarcoma (Table 4).

Downregulated NT5DC2 expression can reduce sarcoma cell proliferation, migration, and invasion

The lentivirus was taken to decrease NT5DC2 expression in sarcoma cells, and the transfection efficiency was confirmed by GFP staining and mRNA expression, as shown in Fig. 7A. EdU and colony formation assays found that downregulating NT5DC2 expressions can obviously suppress the cell proliferation ability, as shown in Fig. 7B, C. Moreover, the wound healing and transwell assays further revealed that decreased NT5DC2 expression can obviously inhibit cell migration and invasion ability in HT1080 and SW872 cells (Fig. 8).

Downregulated NT5DC2 expression can promote cell apoptosis and arrest the G1 cell cycle

The cell apoptosis and cell cycle changes in sarcoma cells after downregulating NT5DC2 expression were detected by flow cytometry assay. The results found that downregulating NT5DC2 expression can lead to a notable increase in sarcoma cell apoptosis rates (Fig. 9A). In addition, cell cycle detection observed that the cell cycle is arrested in G1 stage in sarcoma cells after downregulating NT5DC2 expression (Fig. 9B).

Downregulated NT5DC2 expression suppresses tumor growth in vivo

The xenograft tumor model was used to investigate the effects of NT5DC2 expression changes in tumor growth *in vivo*. The results showed that downregulating NT5DC2 expression can reduce tumor weight and tumor volume *in vivo*, as shown in Fig. 9C.

NT5DC2 promotes sarcoma progression by regulating the ECM-interaction pathway

As shown in Fig. 10, the GO:KEGG analysis of NT5DC2-related genes in TCGA-sarcoma database found that extracellular structure organization and extracellular matrix organization pathways are enriched in GO:BP (Biological Process). Collagen-containing extracellular matrix and extracellular matrix component are enriched in GO;CC (Cellular Component). Extracellular matrix structural constituent and extracellular matrix structural constituent conferring tensile strength are enriched in GO:MF (Molecular Function). Furthermore, the KEGG analysis showed that the ECM-receptor interaction pathway is enriched which containing COL1A1, COL1A2, COL6A1, COL6A2, COL6A3, IBSP, SDC1, and ITGA10. Subsequently, we investigated the expression of these genes in sh-NC and sh-NT5DC2 groups in sarcoma cells, which showed that knockdown of the NT5DC2 gene led to the downregulate expression of COL1A1, COL1A2, COL6A1, COL6A2, COL6A3, IBSP, SDC1, ITGA10 genes, implicating NT5DC2 in the regulation of the ECM-receptor interaction pathway (Fig. 11).

Discussion

Soft tissue sarcoma is a compositional complex and highly heterogeneous mesenchymal malignancy which happened in all parts of the human body, at all ages, with a wide variety and different biological behaviors [18]. Despite extensive research efforts, the etiology of soft tissue sarcoma remains elusive and due to the limited efficacy of chemotherapy and radiotherapy in soft tissue sarcoma patients, surgery is still the main treatment [19,20]. Meanwhile, soft tissue sarcoma has high recurrence and lung metastasis rates, and patients with advanced-stage soft tissue sarcoma cannot tolerate surgery, chemotherapy or radiotherapy [21,22]. These challenges result in a poor prognosis in soft tissue sarcoma patients. Our study dedicated to finding a novel therapeutic target in soft tissue sarcoma which may improve patients' outcomes.

NT5DC2 belongs to the NT5DC family which plays an important role

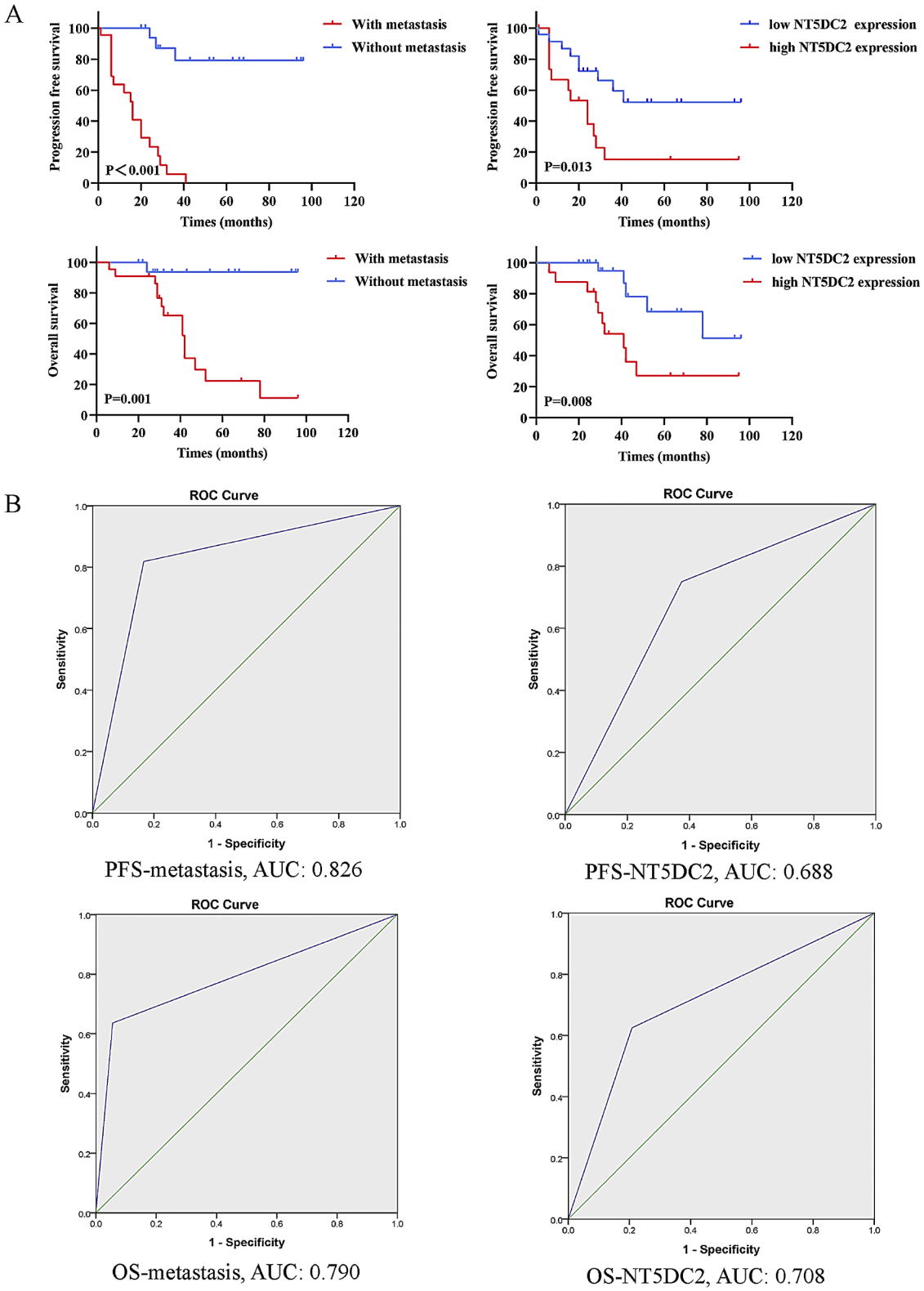

Fig. 6. The associations between NT5DC2 expression and sarcoma patient's survival and it's diagnostic values. (A) Significant differences in PFS and OS were observed in tumor metastasis and NT5DC2 expression status; (B) The ROC curves of tumor metastasis and NT5DC2 expression in PFS and OS in soft tissue sarcoma patients.

Table 3
The univariate analysis of NT5DC2 for soft tissue sarcoma patient survival.

Clinicopathological Data	Case Number	Progression-Free Survival (Months)				Overall Survival (Months)			
		Mean	SD	95 % CI	P value	Mean	SD	95 % CI	P value
Gender									
Male	17	34.412	9.471	15.849–52.975	0.047	54.875	8.21	38.784–70.966	0.184
Female	23	56.697	8.791	39.467–73.928		71.065	7.83	55.718–86.412	
Age (years)									
>60	16	51.622	10.279	31.476–71.768	0.306	67.278	9.814	48.042–86.515	0.452
≤60	24	42.555	8.527	25.842–59.269		60.935	7.131	46.958–74.912	
Tumor size (cm)									
>8	19	29.185	5.242	18.911–39.485	0.072	48.006	6.068	36.113–59.899	0.015
≤8	21	59.600	9.696	40.595–78.605		77.510	8.006	61.818–93.203	
Tumor location									
Thigh	16	40.076	9.608	21.244–58.909	0.325	56.946	9.471	38.382–75.509	0.326
others	24	52.546	8.825	35.250–69.843		68.143	7.092	54.242–82.044	
Metastasis									
Yes	22	17.481	3.209	11.191–23.771	<0.001	46.796	6.096	34.847–58.745	0.001
No	18	81.656	7.412	67.130–96.183		91.5	4.357	82.960–100.040	
Tumor depth									
Deep	30	47.872	7.802	32.580–63.164	0.822	62.845	7.191	48.751–76.938	0.673
Superficial	10	48.844	12.731	23.891–73.797		66.403	9.975	46.852–85.955	
Recurrence									
Yes	17	36.664	9.218	18.596–54.732	0.081	64.008	8.781	46.797–81.219	0.913
No	23	55.447	9.098	37.614–73.280		61.193	8.116	45.286–77.099	
NT5DC2 expression									
High	16	31.200	8.450	14.639–47.761	0.013	48.028	8.368	31.626–64.429	0.008
Low	24	59.319	8.876	41.923–76.716		76.066	7.053	62.242–89.890	

Table 4
The multivariate analysis of NT5DC2 for soft tissue sarcoma patient survival.

Characteristics	Comparison	Progression-Free Survival (Months)			Overall Survival (Months)		
		HR	95 %CI	P value	HR	95 %CI	P value
Metastasis	With *vs* without	14.748	4.096–53.108	<0.001	12.395	1.593–96.460	0.016
NT5DC2	High *vs* low	2.743	1.043–7.218	0.041	3.322	1.063–10.383	0.039

in various tumors. Guo et al. [23] demonstrated that NT5DC2 knockdown could obviously suppress the cell viability, tumor-sphere formation, tumorigenesis, and prolong animal survival in glioma. In pulmonary adenocarcinoma, high expression of NT5DC2 protein is closely related to poor survival status, and NT5DC2 suppression can restrain cell proliferation, induce cell apoptosis, tumorigenesis and hepatic metastasis in lung cancer [24]. Similarly, Zhu et al. [25] found that the knockdown of NT5DC2 suppresses colorectal carcinoma progression by repressing angiogenesis, metastasis, and recruitment of tumor-associated macrophage via the VEGF signaling pathway. While, the roles of NT5DC2 in soft tissue sarcoma progression were undefined. In the current study, we found that NT5DC2 is overexpressed in soft tissue sarcoma tissues, and high expression of NT5DC2 is closely associated with poor prognosis in soft tissue sarcoma patients. Moreover, downregulating NT5DC2 expression can suppress soft tissue sarcoma cell proliferation, migration, invasion, promote cell apoptosis, and arrest the G1 cell cycle.

To further elucidate the potential mechanism involved in the effect of NT5DC2 in soft tissue sarcoma. The KEGG analysis showed that ECM-receptor interaction pathway is significantly enriched in NT5DC2-related genes in soft tissue sarcoma, and previous studies have not reported the associations between NT5DC2 and the ECM-receptor interaction pathway. Notably, ECM-receptor interaction pathway has been implicated in various aspects of tumor biology, including tumor shedding, adhesion, degradation, movement and proliferation [26,27]. ECM consists of a complex mixture of structural and functional macromolecules including fibrous protein and glycosaminoglycans [28,29]. Specific interactions between tumor cells and ECM macromolecules can promote the progression of various tumors. Zhang et al. [30] reported that the activation of the ECM receptor interaction pathway can promote hepatocellular carcinoma proliferation and invasion. In breast cancer, the ECM-receptor interaction pathway plays an important role in cancer progression and metastasis [31]. The important functions of the ECM-receptor interaction pathway in sarcoma have also been confirmed. Starzer et al. [26] demonstrated that tumor DNA methylation profiles related to anti-PD1 immune checkpoint inhibitor monotherapy in sarcoma patients through an ECM-receptor interaction pathway. Meanwhile, the study also reported that the ECM-receptor interaction pathway is involved in the progression of synovial sarcoma [32]. Consists with these studies, our present study showed that NT5DC2-related genes are enriched in the ECM-receptor interaction pathway which including COL1A1, COL1A2, COL6A1, COL6A2, COL6A3, IBSP, SDC1 and ITGA10. Knockdown the expression of NT5DC2 lead to the decreased expression of these genes. Certainly, finding novel therapeutic targets have always been a hot topic in soft tissue sarcoma research, and some effective targeted therapeutic targets will be discovered in the next five years. Nevertheless, several limitations must be acknowledged in our present study. These include the relatively small case numbers and limited follow-up times of soft tissue sarcoma patients, and multi-center study of NT5DC2 functions in soft tissue sarcoma will be more convincing.

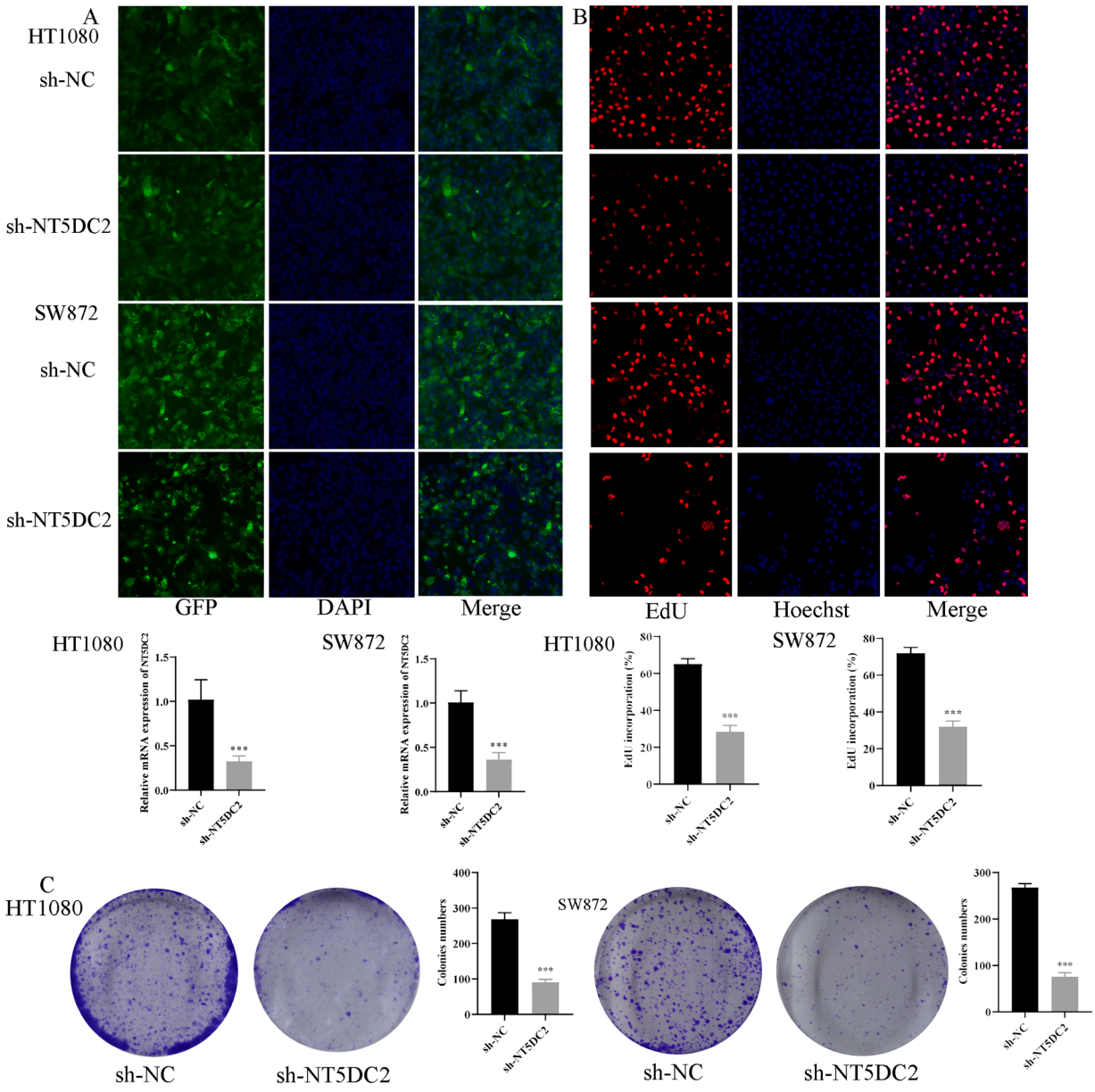

Fig. 7. Downregulated NT5DC2 expression suppressed the proliferation ability in HT1080 and SW872 cells. (A) The transfection efficiency of sh-NC and sh-NT5DC2 lentivirus in HT1080 and SW872 cells; (B) EdU assay showed that knockdown of NT5DC2 significantly restrain the proliferation ability in HT1080 and SW872 cells; (C) Colony formation assay found that knockdown of NT5DC2 remarkably reduce cell colony formation ability in HT1080 and SW872 cells.

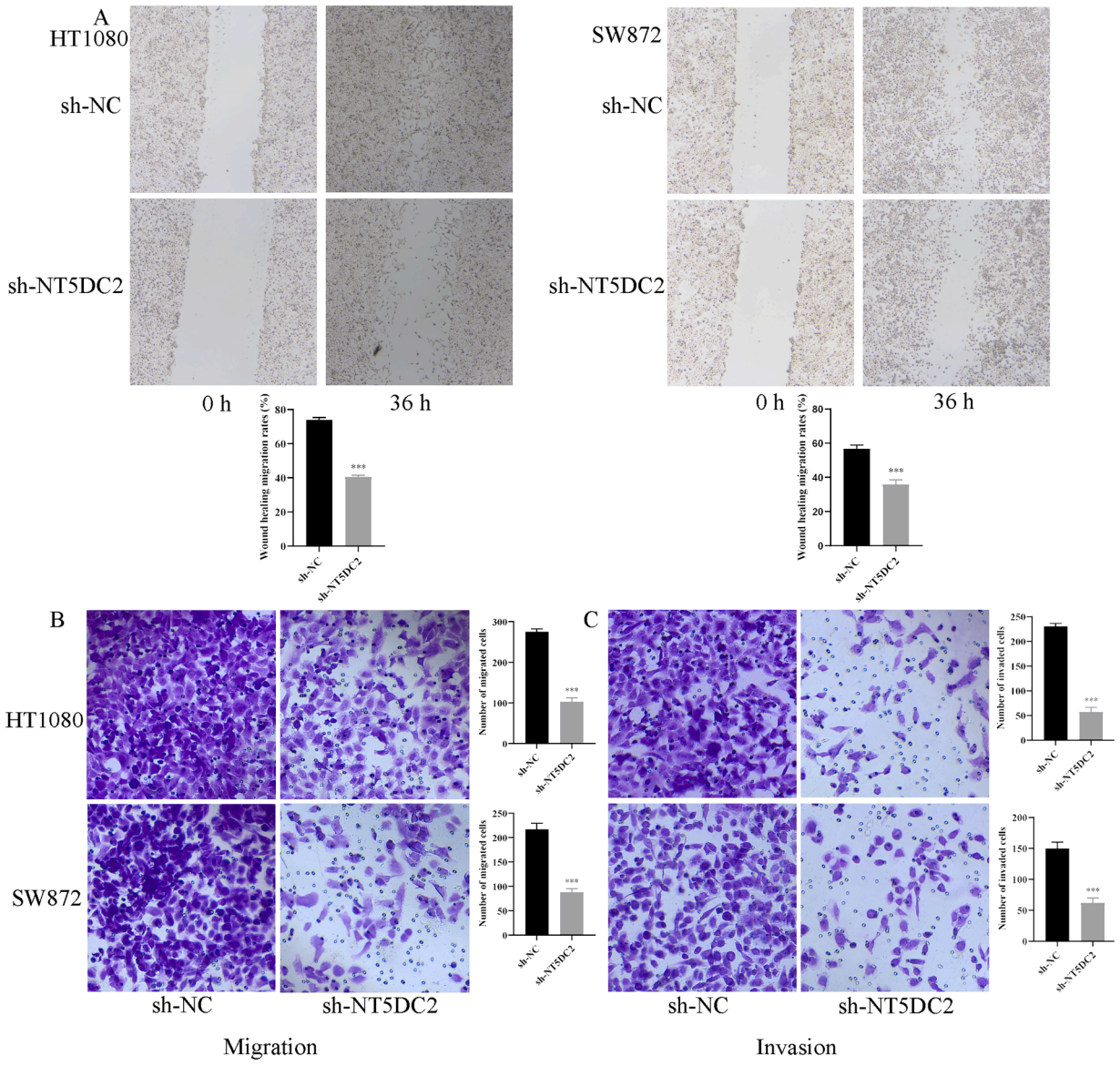

Fig. 8. Downregulated NT5DC2 expression suppressed the migration and invasion ability in HT1080 and SW872 cells. (A) The wound healing detection found that knockdown of NT5DC2 expression obviously decreased the wound healing migration rates in HT1080 and SW872 cells; (B) The migration assay showed that downregulate NT5DC2 expression restrain the migration ability in HT1080 and SW872 cells; (C) The invasion assay revealed that suppress NT5DC2 expression can reduce the invasion ability in HT1080 and SW872 cells. ***$P < 0.001$.

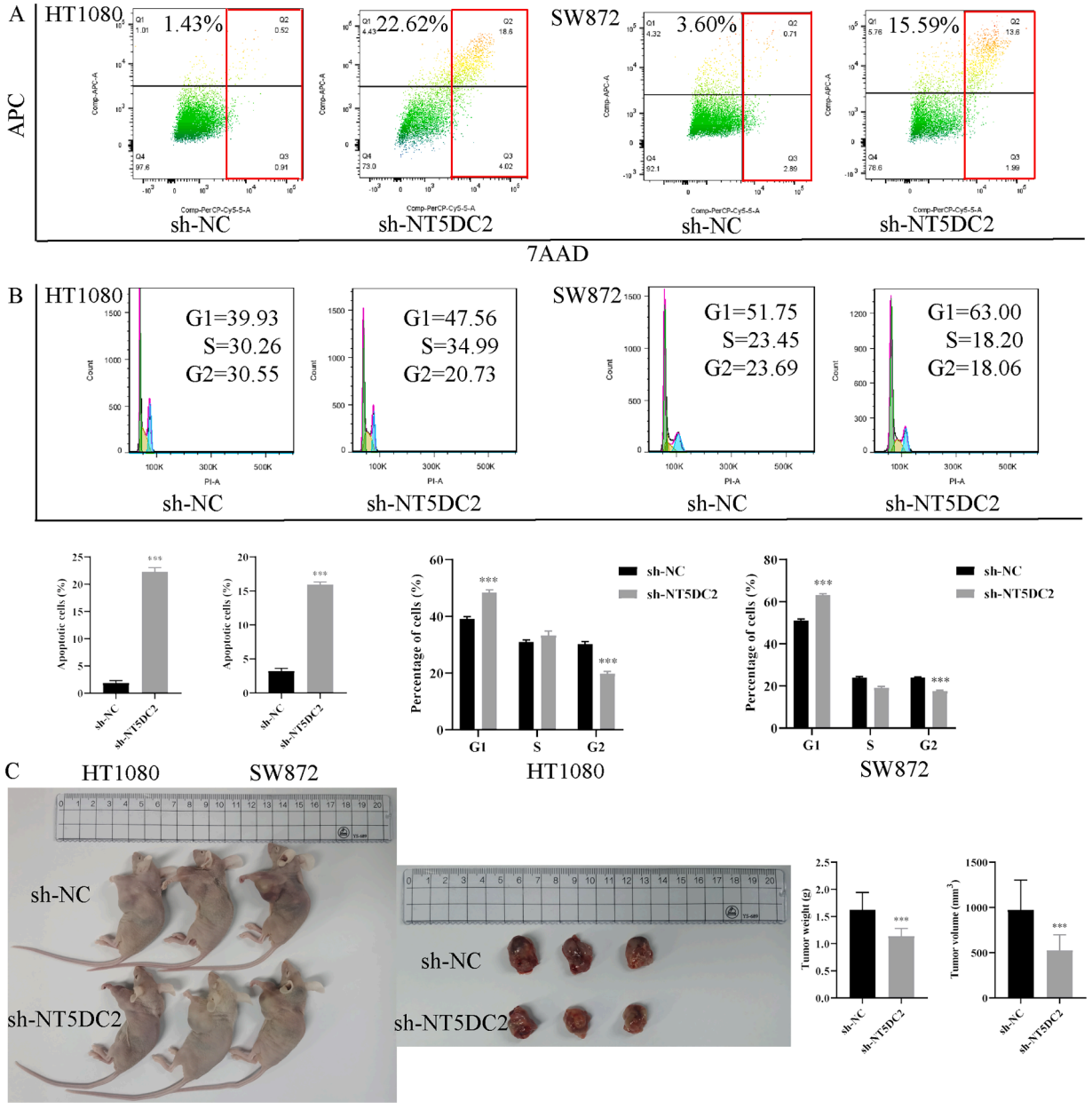

Fig. 9. Knockdown of NT5DC2 expression promoted cell apoptosis, and arrest G1 cell cycle in HT1080 and SW872 cells, and suppressed tumor growth *in vivo*. (A) The apoptosis detection assay showed that cell apoptosis rates in HT1080 and SW872 cells is significantly increased in sh-NT5DC2 group in HT1080 and SW872 cells; (B) The cell cycle detection assay found that downregulate NT5DC2 expression in HT1080 and SW872 cells can arrest cell cycle in G1 stage; (C) The xenograft tumor model results showed that knockdown of NT5DC2 can suppress tumor growth *in vivo*. $^{***}P < 0.001$.

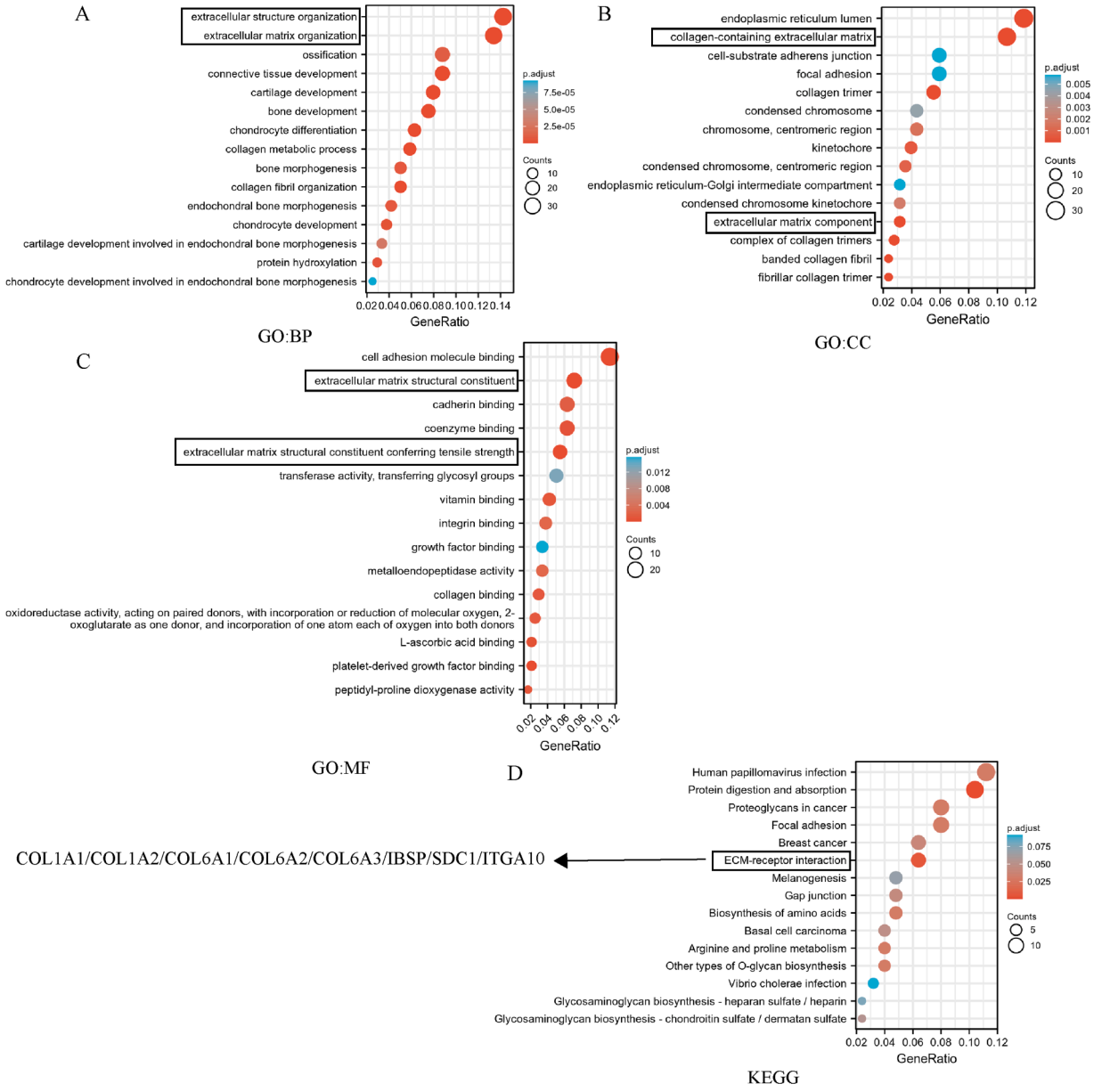

Fig. 10. The GO:KEGG enrichment analysis in NT5DC2 related genes in TCGA-sarcoma. (A) The GO:BP analysis showed that extracellular structural organization and extracellular matrix organization are enriched; (B) The collagen-containing extracellular matrix and extracellular matrix component are enriched in GO:CC pathway; (C) Extracellular matrix structural constituent and extracellular matrix structural constituent tensile strength are enriched in GO:MF pathway; (D) The KEGG pathway detection found that ECM-receptor interaction pathway is enriched.

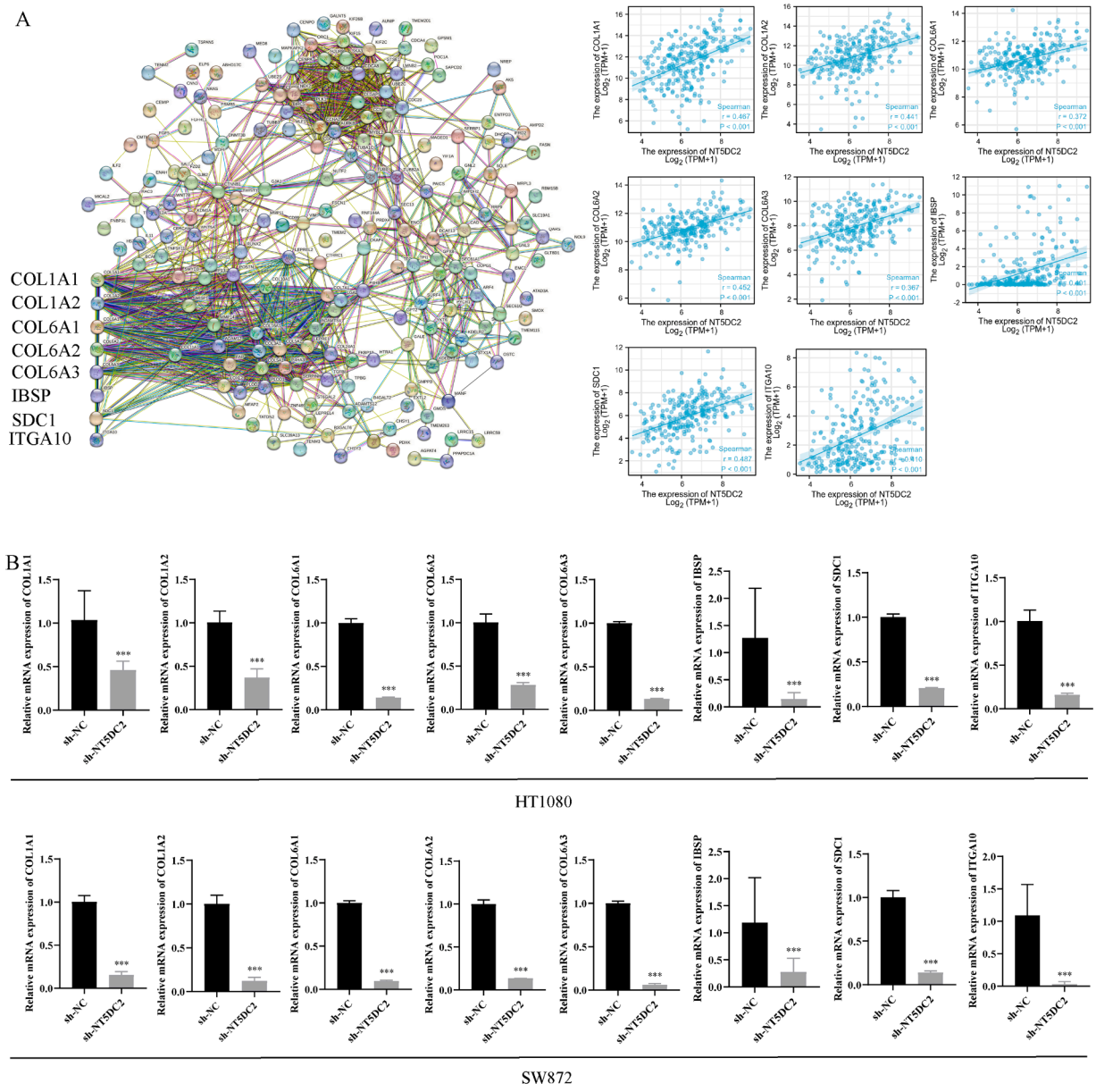

Fig. 11. NT5DC2 plays its effects through regulating ECM-receptor interaction pathway changes in soft tissue sarcoma. (A) The association between NT5DC2 and ECM-receptor interaction pathway related genes (COL1A1, COL1A2, COL6A1, COL6A2, COL6A3, IBSP, SDC1, ITGA10); (B) The RT-qPCR analysis showed that the expression levels of COL1A1, COL1A2, COL6A1, COL6A2, COL6A3, IBSP, SDC1, ITGA10 are significantly reduced in sh-NT5DC2 group compared to sh-NC group in HT1080 and SW872 cells. $^{***}P < 0.001$.

Conclusion

Our study revealed that NT5DC2 protein is overexpressed in soft tissue sarcoma and could be an independent prognostic biomarker in soft tissue sarcoma. Knockdown of NT5DC2 expression exerts a multi-faceted inhibitory effect on sarcoma cell progression via the ECM-receptor interaction pathway. In general, our present study may provide a novel diagnostic biomarker and therapy target in soft tissue sarcoma.

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Ethics statement

It is approved by the Ethics Committee of Shanghai Tenth People's Hospital, Shanghai, China.

Data availability statement

The original data presented in the study are included in the article, further inquiries can be directed to the corresponding author.

CRediT authorship contribution statement

Zhen Huang: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Enjie Xu:** Software, Investigation, Data curation. **Xiaolong Ma:** Investigation, Data curation. **Yongjie Wang:** Investigation, Data curation. **Jiazhuang Zhu:** Investigation, Data curation. **Kunpeng Zhu:** Investigation, Funding acquisition, Data curation. **Jianping Hu:** Investigation, Funding acquisition, Data curation. **Chunlin Zhang:** Supervision, Resources, Project administration, Funding acquisition, Conceptualization, Investigation, Funding acquisition, Data curation.

Declaration of competing interest

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

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