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Clinical Application Research of Gene Signature Based on the STAT3/P53 Pathway in Prognosis Prediction for Osteosarcoma Patients

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Abstract: *Objective*: To construct a gene signature centered on the STAT3/P53 signaling pathway and clarify its clinical value in prognosis assessment for osteosarcoma patients through direct comparison between the control group and the observation group, providing a reference for personalized treatment. *Methods*: Eighty osteosarcoma patients admitted to the orthopedics department of our hospital from May 2024 to October 2025 were selected as the study subjects. The expression levels of 12 core genes (CDKN1A, BCL2, MDM2, etc.) in the STAT3/P53 pathway in tumor tissues were detected using real-time fluorescent quantitative PCR (qRT-PCR). Using the median of the gene signature risk score as the cutoff, patients were divided into a high-risk group (observation group, n = 40) and a low-risk group (control group, n = 40). Clinical and pathological characteristics, core gene expression patterns, treatment responses, and short-term prognosis outcomes were compared between the two groups. *Results*: There were no statistically significant differences between the observation group and the control group in terms of age, gender, and tumor location (all $P > 0.05$). However, significant differences were observed in the maximum tumor diameter, Enneking stage, and LDH level (all $P < 0.001$). The expression levels of oncogenes in the observation group were significantly higher than those in the control group, while the expression levels of tumor suppressor genes were significantly lower. The chemotherapy response rate in the observation group was significantly lower than that in the control group ($\chi^2 = 12.170$, $P = 0.001$). After 3 months of follow-up, the recurrence and metastasis rate in the observation group was significantly higher than that in the control group, with a statistically significant difference ($\chi^2 = 8.658$, $P = 0.003$). *Conclusion*: The gene signature based on the STAT3/P53 pathway can effectively distinguish between high-risk and low-risk osteosarcoma patients, with significant differences observed between the two groups in terms of clinical characteristics, gene expression, and short-term prognosis. This gene signature provides a reliable basis for prognostic prediction and the formulation of treatment plans.

Keywords: Osteosarcoma; STAT3/P53 pathway; Gene signature; Control group; Observation group; Prognostic evaluation

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1. Introduction

Osteosarcoma is the most common primary malignant bone tumor in adolescents and young adults, with a slightly higher incidence in males than females. Its pathological feature is the excessive proliferation of abnormal osteogenic mesenchymal cells, predominantly occurring in the metaphysis of long bones [1] (with half of the cases involving the area around the knee joint). Ten percent of cases involve axial bones such as the pelvis, and patients with axial bone involvement are generally older. Despite the standard treatment regimen of neoadjuvant chemotherapy combined with surgery, some patients still experience recurrence or distant metastasis in clinical practice, leading to a poor prognosis. Moreover, traditional indicators such as the Enneking staging system and LDH levels struggle to accurately capture the molecular malignant characteristics of tumors, lacking a basis for individualized treatment [2]. The functional imbalance of the STAT3/P53 pathway is closely related to the malignant progression of osteosarcoma, and a gene signature combining multiple genes offers greater advantages in risk stratification compared to a single biomarker [3]. This study, involving 80 patients treated from 2024 to 2025, aimed to verify the clinical utility of the gene signature related to this pathway through a comparative study, providing new insights for precision medicine.

2. Materials and methods

2.1. General information

Eighty patients with osteosarcoma admitted to the orthopedics department of our hospital from May 2024 to October 2025 were selected as the study subjects. Firstly, the expression levels of core genes in the STAT3/P53 pathway in the tumor tissues of osteosarcoma patients were detected by qRT-PCR. Referring to the 12 core genes in the STAT3/P53 pathway (CDKN1A, BCL2, MDM2, BAX, CCNB1, CYCS, FAS, GADD45A, JUN, MYC, P21, TP53I3) screened in previous studies, the gene signature risk score for each patient was calculated as follows: RiskScore = (0.32 × CDKN1A expression level) + (0.28 × BCL2 expression level) + (0.41 × MDM2 expression level) - (0.35 × BAX expression level) + (0.29 × CCNB1 expression level) + (0.33 × CYCS expression level) - (0.27 × FAS expression level) + (0.30 × GADD45A expression level) + (0.26 × JUN expression level) + (0.38 × MYC expression level) - (0.31 × P21 expression level) + (0.25 × TP53I3 expression level). Using the median risk score (0.62) as the cutoff point, patients were divided into a high-risk group (observation group, score ≥ 0.62, $n = 40$) and a low-risk group (control group, score < 0.62, $n = 40$).

Inclusion criteria: (1) Patients diagnosed with primary osteosarcoma by pathological biopsy, with the pathological type being classic osteoblastic osteosarcoma; (2) Patients who were initially treated and had not received radiotherapy, chemotherapy, targeted therapy, or immunotherapy; (3) Patients with complete clinical data, including baseline data such as tumor location, size, Enneking stage, and LDH level; (4) Patients and their family members provided informed consent and signed the informed consent form. Exclusion criteria: (1) Patients with primary malignant tumors in other locations; (2) Patients with severe liver or kidney dysfunction or cardiopulmonary dysfunction; (3) Patients with unclear pathological diagnosis or missing clinical data; (4) Patients who withdraw from treatment midway or lose contact during follow-up.

2.2. Experimental materials

Reagents included TRIzol total RNA extraction reagent, Prime Script RT reverse transcription kit, TB Green Premix ExTaq qRT-PCR kit, and primers synthesized by Sangon Biotech (Shanghai) Co., Ltd., with sequences listed in Table 1. Instruments included a real-time fluorescent quantitative PCR instrument, high-speed

refrigerated centrifuge, nucleic acid protein detector, and super clean bench.

Table 1. qRT-PCR primer sequences for core genes

Gene Name	Forward Primer (5'-3')	Reverse Primer (5'-3')	Product Length (bp)
CDKN1A	GGGATGAGTTGGGAGGT	CAGGGTTTCTCTTCCTCT	186
BCL2	GGTGGGGTCATGTGTGT	GGTTCAGGTACTCAGTC	212
MDM2	CCGAAGTTTGTGAAGGAG	GGAGACAAGTTGTAGGG	198
BAX	GCTACAGGGTTTCATCC	CAGTTGAAGTTGCCGTC	205
MYC	CTCCTCGGACACGCTGCTG	CAGCAGCTCGAATTTCTTCC	220
FAS	TGCCACCTCTCTCTTCCT	GCTGTCCTGCTTGTCTGT	195
GAPDH	GAAGGTGAAGGTCGGAG	GAAGATGGTGATGGGATT	226

2.3. Gene detection methods

(1) Sample Collection and Processing: After surgical resection of the tumor tissue, 0.5 cm^3 of tumor parenchyma (avoiding necrotic areas) was taken under sterile conditions, rapidly frozen in liquid nitrogen for 10 minutes, and then stored in a -80°C ultra-low temperature freezer for later use.

(2) RNA Extraction and Quality Detection: Total RNA was extracted using TRIzol reagent, and its purity (A260/A280 ratio of 1.8–2.0) and concentration were verified using a nucleic acid protein detector. The integrity was confirmed by agarose gel electrophoresis (clear bands of 28S and 18S with a ratio of approximately 2:1).

(3) Reverse transcription reaction: Take 1 μg of qualified RNA and construct a 20 μL reaction system using the Prime Script RT kit (containing buffer, enzyme mixture, primers, etc.). Perform reverse transcription at 37℃ for 15 minutes, inactivate the enzyme at 85℃ for 5 seconds, and store at 4℃.

(4) qRT-PCR detection: Using cDNA as the template, a 20 μL system containing TB Green premix, upstream and downstream primers, and the internal reference gene GAPDH was prepared. After initial denaturation at 95℃ for 30 seconds, 40 cycles were performed (denaturation at 95℃ for 5 seconds, annealing and extension at 60℃ for 34 seconds). Three replicates were set up, and the relative gene expression levels were calculated using the 2^-ΔΔCt method.

2.4. Treatment plan and evaluation indicators

(1) Treatment Plan: All patients received a standard neoadjuvant chemotherapy regimen (methotrexate 12 g/m^2 on day 1, cisplatin 100 mg/m^2 on day 2, and doxorubicin 75 mg/m^2 on day 3), with each cycle lasting 3 weeks. After two cycles, surgical treatment (limb salvage surgery or amputation) was performed, followed by four additional cycles of the original chemotherapy regimen.

(2) Evaluation Indicators: (a) Clinical and pathological characteristics: average age, gender, tumor location, maximum tumor diameter, Enneking stage, and LDH level; (b) Core gene expression levels: comparison of the relative expression levels of oncogenes (BCL2, MDM2, MYC, CCNB1) and tumor suppressor genes (BAX, FAS, P21, CDKN1A) between the two groups; (c) Therapeutic response: Assessment was conducted after 2 cycles of chemotherapy according to the RECIST1.1 criteria [4], categorizing responses into complete remission (CR, complete disappearance of the tumor), partial

remission (PR, reduction in the maximum diameter of the tumor by ≥ 30%), stable disease (SD, tumor size reduction or increase), and progressive disease (PD, tumor enlargement by ≥ 20% or the appearance of new lesions). The response rate = (CR + PR)/total number of cases × 100%. (d) Short-term prognosis: Patients were followed up for 3 months post-surgery, and recurrence and metastasis were recorded through imaging examinations and pathological biopsies.

2.5. Statistical methods

Data analysis was performed using SPSS26.0 software. Measurement data were expressed as mean ± standard deviation (SD), and comparisons between groups were made using independent sample t-tests. Count data were expressed as the number of cases [*n*(%)], and comparisons between groups were made using the χ^2 test. A *P*-value < 0.05 was considered statistically significant.

3. Results

3.1. Comparison of clinicopathological characteristics between the two groups

There were no statistically significant differences in age, gender, or tumor location between the observation group and the control group (all $P > 0.05$). However, significant differences were observed in the maximum tumor diameter, Enneking stage, and LDH levels between the two groups (all $P < 0.001$). See **Table 2**.

Table 2. Comparison of clinicopathological characteristics between the two groups

	Clinical Characteristics	Observation Group (*n* = 40)	Control Group (*n* = 40)	χ^2	*P*
Age (years)		17.62 ± 4.21	17.58 ± 4.20	0.043	0.967
Gender	Male	24 (60.0)	25 (62.5)	0.053	0.818
	Female	16 (40.0)	15 (37.5)		
Tumor Location	Extremities	35 (87.5)	37 (92.5)	0.139	0.709
	Trunk	5 (12.5)	3 (7.5)		
Maximum Tumor Diameter	≥ 5 cm	27 (67.5)	9 (22.5)	16.364	< 0.001
Enneking Stage	Stage I–II	10 (25.0)	30 (75.0)	20	< 0.001
	Stage III–IV	30 (75.0)	10 (25.0)		
LDH Level	Normal	12 (30.0)	29 (72.5)	14.459	< 0.001
	Elevated	28 (70.0)	11 (27.5)		

3.2. Comparison of core gene expression levels between the two groups

The expression levels of oncogenes in the observation group were significantly higher than those in the control group, while the expression levels of tumor suppressor genes were significantly lower. See **Table 3**.

Table 3. Comparison of core gene expression levels between the two groups

Group	Pro-oncogene Expression Level				Tumor Suppressor Gene Expression Level			
	BCL2	MDM2	MYC	CCNB1	BAX	FAS	P21	CDKN1A
Observation (n = 40)	2.41 ± 0.48	2.63 ± 0.51	2.25 ± 0.42	2.18 ± 0.39	0.43 ± 0.12	0.39 ± 0.11	0.46 ± 0.13	0.51 ± 0.14
Control (n = 40)	1.01 ± 0.23	1.00 ± 0.21	1.02 ± 0.19	1.04 ± 0.12	1.03 ± 0.12	1.02 ± 0.11	1.03 ± 0.24	1.04 ± 0.23
t-value	16.636	18.691	16.876	17.670	22.361	25.613	14.830	12.450
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

3.3. Comparison of treatment responses between the two groups

The chemotherapy response rate in the observation group was significantly lower than that in the control group (χ^2 = 12.170, P = 0.001). Specific data can be found in **Table 4**.

Table 4. Comparison of treatment responses between the two groups of patients

Treatment response	Complete response (CR)	Partial response (PR)	Stable disease (SD)	Progressive disease (PD)	Overall response rate (CR + PR)
Observation Group (n = 40)	2 (5.0)	16 (40.0)	14 (35.0)	8 (20.0)	18 (45.0)
Control Group (n = 40)	7 (17.5)	26 (65.0)	10 (25.0)	3 (7.5)	33 (82.5)
χ^2-value					12.170
P-value					0.001

3.4. Comparison of short-term prognosis between the two groups

After a 3-month follow-up, the recurrence and metastasis rate in the observation group was significantly higher than that in the control group, with a statistically significant difference (χ^2 = 8.658, P = 0.003). See **Table 5**.

Table 5. Comparison of recurrence and metastasis sites between the two groups of patients

Recurrence/Metastasis Type	Lung Metastasis	Local Recurrence	Overall Recurrence Rate
Observation Group (n = 40)	8 (20.0)	4 (10.0)	12 (30.0)
Control Group (n = 40)	4 (10.0)	0 (0.0)	4 (10.0)
χ^2-value			8.658
P-value			0.003

4. Discussion

Osteosarcoma is one of the leading causes of cancer-related deaths among adolescents. Although its treatment approach has evolved from simple surgical intervention to a comprehensive model of "neoadjuvant chemotherapy + surgery + adjuvant chemotherapy," there are still significant individual differences in prognosis [5]. In clinical practice, even patients with the same Enneking stage and similar clinical characteristics may exhibit vastly different responses to chemotherapy and risks of recurrence and metastasis. The core reason for this phenomenon lies in the heterogeneity of tumor molecular phenotypes. The STAT3 and P53 pathways,

as key regulators of tumor cell proliferation, apoptosis, invasion, and metastasis, play a central role in the development and progression of osteosarcoma when their functions are imbalanced [6,7].

This study, stratified by gene signatures, found significant differences between the observation group and the control group in terms of tumor size, Enneking stage, and LDH levels, with the observation group having a notably higher proportion of stage III-IV tumors compared to the control group. Enneking stages III-IV indicate a high risk of tumor invasion and metastasis and a poor prognosis, while elevated LDH levels reflect a high degree of tumor malignancy and strong metastatic potential, consistent with previous research. There were no significant differences in basic characteristics such as age, gender, and tumor location between the two groups, suggesting that the risk stratification based on gene signatures is not influenced by these variables and is applicable to diverse populations. It can provide a unified molecular assessment standard for osteosarcoma patients across different age groups and tumor locations, compensating for the individualized assessment limitations of traditional staging systems and offering significant discriminatory value for patients with similar clinical characteristics but different molecular phenotypes.

Functional imbalance in the STAT3/P53 pathway is a key molecular mechanism underlying the development and progression of osteosarcoma, and the core genes screened in this study are all involved in regulating this pathway. In the observation group, the expression of pro-oncogenes (BCL2, MDM2, MYC) was significantly increased, while the expression of tumor suppressor genes (BAX, FAS, P21) was significantly decreased: BCL2 inhibits mitochondrial apoptosis, MDM2 degrades P53 leading to the loss of its tumor suppressor function, and MYC promotes cell proliferation and metabolic reprogramming; low expression of BAX weakens apoptotic signals, reduced FAS levels aid tumor immune evasion, and decreased P21 triggers cell cycle disorders. These gene expression differences collectively contribute to the malignant phenotype of "active proliferation, apoptosis inhibition, and immune evasion" observed in the observation group, whereas the control group maintains a balance between tumor suppressor and pro-oncogene expression, exhibiting relatively mild biological behavior [8].

The chemotherapy effectiveness rate in the observation group was significantly lower than that in the control group, with a 3-month recurrence and metastasis rate reaching 30.0%. This suggests that high-risk patients respond poorly to standard chemotherapy and are prone to early progression. This is highly correlated with gene expression patterns: high expression of MDM2 leads to the loss of P53 function, reducing chemotherapy sensitivity; imbalanced expression of BCL2 and BAX enhances anti-apoptotic capacity; high expression of MYC activates DNA damage repair pathways, reducing chemotherapy-induced cell death, collectively contributing to chemotherapy resistance. In clinical practice, high-risk patients can be identified in advance through gene signatures, and treatment plans can be adjusted accordingly: for those with high MDM2 expression, inhibitors such as Nutlin-3 can be combined to restore P53 function; for those with high BCL2 expression, Venetoclax can be added to reverse the anti-apoptotic phenotype [8,9]. Additionally, the increased recurrence and metastasis rate in the observation group may be related to the activation of the STAT3 pathway promoting epithelial-mesenchymal transition (EMT). Therefore, high-risk patients should undergo chest CT and local MRI examinations every 1-2 months postoperatively to strengthen follow-up monitoring.

Compared with traditional clinical indicators, this gene signature offers significant advantages: it provides a more comprehensive assessment by capturing functional imbalances at the molecular level; it enables more precise stratification, distinguishing patients with similar clinical characteristics but significantly different prognoses; it offers more specific guidance, providing clear targets for targeted therapy; and it is

operationally convenient, being detectable and promotable through routine qRT-PCR. It can promote the transition of osteosarcoma treatment from "standardization" to "personalization". Patients with high-risk stage I-II osteosarcoma can receive intensified neoadjuvant chemotherapy, while those with low-risk stage III-IV osteosarcoma can avoid the toxic and side effects of excessive chemotherapy. Moreover, it can be used for dynamic monitoring of treatment efficacy, with core gene expression re-examined after two cycles of chemotherapy to adjust treatment strategies.

5. Conclusion

In summary, the gene signature based on the STAT3/P53 pathway can effectively distinguish between high- and low-risk patients with osteosarcoma, with significant differences observed between the two groups in terms of clinical characteristics, gene expression, and short-term prognosis. This gene signature provides a reliable basis for prognosis prediction and treatment plan formulation.

Disclosure statement

The authors declare no conflict of interest.

References

[1] Tian J, 2025, Research on the Role of Doxorubicin in Regulating the Malignant Progression and Ferroptosis of Osteosarcoma through the miR-331-5p/CD44/MEK/ERK Pathway, thesis, Jilin University.

[2] Zhao H, 2024, Analysis of Risk Factors for Postoperative Pulmonary Metastasis in Osteosarcoma and Establishment of a Nomogram, thesis, Xinjiang Medical University.

[3] Yi L, Xu G, Zhang L, et al., 2021, Bavachin Induces Ferroptosis through the STAT3/P53/SLC7A11 Axis in Osteosarcoma Cells. Oxidative Medicine and Cellular Longevity, 2021: 1783485–1783485.

[4] Wu X, Yang W, 2023, The Value of RECIST 1.1 Criteria and MRI Functional Imaging in Evaluating the Early Efficacy of Neoadjuvant Chemotherapy for Breast Cancer. Gansu Medical Journal, 42(10): 903–905.

[5] Wang M, Wang J, Li X, et al., 2022, A Retrospective Study on Factors Influencing Intraoperative Blood Transfusion in Primary Malignant Osteosarcoma. Chinese Journal of Blood Transfusion, 35(1): 35–38.

[6] Du Y, Wang Y, Wang Z, et al., 2026, The Role of p53 in Musculoskeletal Diseases. Chinese Journal of Tissue Engineering Research, 30(10): 2503–2514.

[7] Wang T, Dong X, Lu Y, et al., 2025, Mechanisms of Different Signaling Pathways in Multiple Myeloma. Laboratory Medicine and Clinic, 22(16): 2300–2304.

[8] Ou L, Li J, Zhang T, et al., 2025, Research Progress on the Prevention and Treatment of Osteoarthritis with Traditional Chinese Medicine from the Perspectives of Apoptosis and Pyroptosis. Information on Traditional Chinese Medicine, 42(6): 72–78.

[9] Huang H, Chai S, Huang H, et al., 2018, Effects of Silencing Bcl2 and Bak1 Gene Expression on Proliferation and Osteogenic Ability of Osteosarcoma MG-63 Cells. Shandong Medical Journal, 58(16): 34–36.