Journal of Bone Oncology 23 (2020) 100299

Contents lists available at ScienceDirect

Journal of Bone Oncology

journal homepage: www.elsevier.com/locate/jbo

Effects of different levels of TGF-β expression and tumor cell necrosis rates in osteosarcoma on the chemotherapy resistance of osteosarcoma

Ling Zhou[a], Jiadai Tang[a], Fengdi Hu[a], Yedan Liao[a], Rong Li[a], Yonghong Zhou[b], Zhihong Yao[c], Zhengqin Geng[a], Zuozhang Yang[c,*], Xueqi Zhang[a], Lin Xie[a,*]

[a] Department of Gastrointestinal Oncology, The Third Affiliated Hospital of Kunming Medical University, Cancer Hospital of Yunnan Province, No. 519 Kunzhou Road, Kunming, Yunnan, China

[b] Department of Palliative Medicine, The Third Affiliated Hospital of Kunming Medical University, Cancer Hospital of Yunnan Province, No. 519 Kunzhou Road, Kunming, Yunnan, China

[c] Bone and Soft Tissue Tumors Research Center of Yunnan Province, Department of Orthopaedics, The Third Affiliated Hospital of Kunming Medical University, Cancer Hospital of Yunnan Province, No. 519 Kunzhou Road, Kunming, Yunnan, China

ARTICLE INFO

Keywords:
TGF-β
Tumor cell necrosis rate
Osteosarcoma
Chemotherapy resistance

ABSTRACT

Purpose: The clinical significance of transforming growth factor β (TGF-β) and tumor cell necrosis rate (TCNR) in the expression of osteosarcoma and its effects of chemotherapy resistance on osteosarcoma were explored.

Patients and methods: 94 cases of neoadjuvant chemotherapy osteosarcoma patients at the Third Affiliated Hospital of Kunming Medical University between January 2014 and January 2019 were collected. Samples tested for TGF-β were collected before chemotherapy, the tumor cell necrosis rate of pathological samples before and after chemotherapy was determined. Others analyzed covariates included 12 prognostic factors that may be associated with chemotherapy resistance in previous studies: age, BMI, initial diagnosis time (The time from symptom onset to first medical attention), KPS score, initial tumor size, lymphocytes/leukocytes rate (LWR), neutrophils/lymphocytes rate (NLR), albumin, aspartate transaminase (AST), low density lipoprotein (LDL), blood urea nitrogen (BUN), alkaline phosphatase (ALP), the endpoints included progression-free survival (PFS) and overall survival (OS), response evaluation criteria in solid tumours by RECIST guideline (version 1.1).

Result: 1. A total of 94 cases were examined for expression of TGF-β in pathological specimens, 45 cases were TGF-β high expression (47.9%) and 49 cases were TGF-β low expression (52.1%); 2. The BMI, LDL, ALP, NLR in TGF-β high expression group was significantly increased compared to TGF-β low expression group; the Initial diagnosis time, KPS in TGF-β high expression group was significantly decreased compared to TGF-β low expression group, all $P < 0.05$; 3. Effect of chemotherapy was positively with positive cell rate ($P < 0.01$ $r = 0.337$) and TGF-β total score ($P < 0.0001$ $r = 0.635$), while effect of chemotherapy was no correlation with degree of dyeing score ($P > 0.05$); there was significant difference in change from baseline after chemotherapy between TGF-β high expression group and TGF-β low expression group ($P = 0.045$); 4. Median OS 61.4 months in the TGF-β high expression group, median OS 68.1 months in the TGF-β low expression group, one-year survival rate, there was statistically significant difference in two groups ($P = 0.045$); median PFS 44.8 months in the TGF-β high expression group, median PFS 56.2 months in the TGF-β low expression group, There was no statistically significant difference in two groups ($P > 0.05$); 5. A total of 92 cases were examined for TCNR after chemotherapy, 62 were TCNR ≤ 90% (67.4%), 30 were TCNR > 90% (32.6%); 6. the Initial diagnosis time, KPS, in TCNR > 90% group was significantly increased compared to TCNR ≤ 90% group; the initial tumor size, BUN, ALP in TCNR > 90% group was significantly decreased compared to TCNR ≤ 90% group, all $P < 0.05$; 7. TCNR was negatively correlated with the change from baseline after chemotherapy ($P < 0.001$ $r = -0.411$); there was no statistically significant difference between TCNR > 90% group and TCNR ≤ 90% group in change

Abbreviations: TGF-β, transforming growth factor β; TCNR, tumor cell necrosis rate; LWR, lymphocytes/leukocytes rate; NLR, neutrophils/lymphocytes rate; AST, aspartate transaminase; LDL, low density lipoprotein; BUN, blood urea nitrogen; ALP, alkaline phosphatase; PFS, progression-free survival; OS, overall survival; EMT, epithelial-mesenchymal transition

* Corresponding authors at: Bone and Soft Tissue Tumors Research Center of Yunnan Province, Department of Orthopaedics, The Third Affiliated Hospital of Kunming Medical University, Cancer Hospital of Yunnan Province, No. 519 Kunzhou Road, Kunming, Yunnan 650118, China (Z. Yang). Department of Gastrointestinal Oncology, The Third Affiliated Hospital of Kunming Medical University, Cancer Hospital of Yunnan Province, No. 519 Kunzhou Road, Kunming, Yunnan 650118, China (L. Xie).

E-mail addresses: yangzuozhang@163.com (Z. Yang), xielinyanghan@163.com (L. Xie).

https://doi.org/10.1016/j.jbo.2020.100299
Received 7 December 2019; Received in revised form 4 March 2020; Accepted 27 April 2020
Available online 01 June 2020

from baseline after chemotherapy ($P > 0.05$); 8. Median OS 67.8 months in the TCNR > 90% group, median OS 61.7 months in the TCNR ≤ 90% group, there was statistically significant difference between two groups ($P = 0.040$); median PFS 57.4 months in the TCNR > 90% group, median PFS 40.5 months in the TCNR ≤ 90% group, there was statistically significant difference between two groups ($P = 0.036$); 9. TGF-β total score was negatively correlated with TCNR ($P < 0.001$ $r = -0.571$).

Conclusion: The results of this study suggested that the higher expression of TGF-β, the lower expression of TCNR, which more likely to induce chemotherapy resistance among patients with osteosarcoma and lead to poor prognosis.

1. Introduction

Osteosarcoma is the most common primary bone malignancy in adolescents, the combination of surgery and multiagent chemotherapy in recent years has indeed led to a dramatic increase in the survival rate, nowadays overall survival (OS) rates of 70% for patients with localized disease and 30% for those with metastatic disease in developed countries [1]. But some patients are not sensitive to chemotherapy with poor prognosis, the cause of it is mainly due to tumor heterogeneity. The genesis of tumor heterogeneity is closely associated with tumor stem cells, genetic instability, cell competition and stochastic events [2]. One of the current focuses of attention is to explore the factors influencing the drug resistance of osteosarcoma. In recent years, some progress had been made in the study of the mechanism of tumor drug resistance, but the internal connection of mechanism remains to be further explored.

Recent study reported TGF-β might establish an intrinsic link between tumor resistance and metastasis [3], TGF-β could regulate intercellular and intracellular signal networks through autocrine and paracrine modes, affecting cell proliferation, differentiation and apoptosis in the body [4]. Transcription factor Smad3 is a key protein molecule downstream of TGF-β signaling, Smad3 plays a key role in regulating TGF-β to induce EMT and Smad3 transduces the signal from the cytoplasm to the nucleus after binding TGF-β to its receptor [5], nuclear localization of Smad3 would be a good indication of the activation of the TGF-β pathway in tumor cells. Brabletz et al found TGF-β could affect the sensitivity of chemotherapy, Blocking the TGF-β1 signaling pathway can significantly increase the chemotherapy sensitivity of drugs [6]. Lin et al. reported TGF-β1 can induce the expression of miR-202. which can promotes chemotherapy resistance by targeting tumor suppressor PDCD4 [7]. Brunen et al presented data demonstrating that TGF-β plays a key role in chemotherapy resistance in colorectal cancer [8]. However, the affects of TGF expression extent to chemotherapy resistance, and which factors are associated with TGF expression, is not yet know. Immunohistochemistry (IHC) was used in this study to explore the expression of TGF-β in primary osteosarcoma tissues before chemotherapy to evaluate the relationship between the proposed biomarker and clinical parameters, such as overall survival, response to chemotherapy, metastasis or proliferation of the primary tumor, in order to assess the clinical value of TGF-β.

TCNR is believed to have the advantage of being able to be evaluated accurately, which is a gold standard to reflect the sensitivity of osteosarcoma to chemotherapy, to predict tumor outcomes and to guide postoperative chemotherapy [9]. TCNR > 90% showed good response, suggesting that preoperative chemotherapy regimen could be continued, TCNR ≤ 90% showed poor response, suggesting that chemotherapy drugs needs to be changed, measuring by TCNR, we can value patients who are not sensitive to chemotherapy as early as possible and develop individualized treatment plans for their reactivity [9]. Therefore, this study intends to explore the clinical significance of expression of TGF-β and TCNR in osteosarcoma and its effect on the chemotherapy resistance of osteosarcoma, so as to provide sufficient theoretical basis for the development of individualized chemotherapy regimen for osteosarcoma.

2. Material and methods

2.1. Study design and patients

94 cases of neoadjuvant chemotherapy osteosarcoma patients at the Third Affiliated Hospital of Kunming Medical University between January 2014 and January 2019 were collected, all patients underwent surgery (Amputations or Tumor resection) after neoadjuvant chemotherapy. All patients signed the informed consent. The inclusion criteria were as follows: (1) There is a clear basis for pathological diagnosis; (2) Patients receiving neoadjuvant chemotherapy before surgery; (3) No double carcinoma; (4) Case data, imaging data and pathological specimens before and after neoadjuvant chemotherapy were kept intact; (5) No serious bone-related disease other than osteosarcoma may affect pathological specimens. This study was approved by the Ethics Committee of the Third Affiliated Hospital of Kunming Medical University. This trial was conducted in accordance with the Declaration of Helsinki. We confirm that patient data confidentiality was maintained. All osteosarcoma obey Ennecking surgical staging [10].

2.2. General condition

The analyzed covariates included 12 prognostic factors that may be associated with chemotherapy resistance in previous studies: age, BMI, initial diagnosis time, KPS score, initial tumor size, LWR, NLR, albumin, AST, LDL, BUN, ALP, the endpoints included PFS and OS. Chemotherapy response evaluation criteria in solid tumours by RECIST version 1.1: complete response (CR)-target lesion completely disappeared, partial response (PR)-change from baseline decrease ≥30%, progress disease (PD)-change from baseline increase ≥20%, stable disease (SD) was between PD and PR. PD is considered to be chemotherapy resistant.

2.3. Pathological covariates

2.3.1. TGF-β IHC methods

Samples tested for TGF-β were collected before chemotherapy. P Paraffin sections were dewaxed, hydrated and rinsed with distilled water, According to the requirements of first antibody TGF-β1 (Yunnan HAOZE company, China) to tissue antigen repair; the slices were dripped with endogenous peroxidase blocker, rinse with PBS then drop the first antibody on the section, after incubate it at room temperature rinse it with PBS; Primary Antibody Enhancer (JINYU company, China) was added to the section and incubated at room temperature, then washed with PBS; secondary antibody-HRP Polymer (Yunnan HAOZE company, China) reagent was added, incubated at room temperature and washed.

Interpretation standard: Each pathological section was randomly selected from the tumor center and the invasion front area to read 5 non-repetitive fields of equal area: positive cell rate < 5% was considered negative then record 0; positive cell rate > 5% was considered positive: 6–25% is 1 points, 25–50% is 2 points, 50–75% is 3 points, > 75 is 4 points. Degree of dyeing score standard: no color is 0 points, pale yellow is 1 points, yellow or brown is 2 points, tan is 3

points. The product of positive cell rate and degree of dyeing score was taken as the total score, calculate the median of all the total scores as the cutoff value, greater than or equal to median is high expression, lower than the median is a low expression. The above results were observed and confirmed by at least two professional pathologists.

2.4. TCNR detection

Representative pathological sections of biopsy specimens before chemotherapy were selected, five fields were randomly selected under the microscope, tumor cells were counted [11], and their mean value was M as the base. After the neoadjuvant chemotherapy, the surgically resected specimens were cut from multiple sections after a comprehensive general examination, and at least 3 samples were selected from different materials, the specimens were fixed with 10% formalin for 24 h and decalcified with 5% nitric acid for 24 h, the specimens were rinsed by water and the paraffin tissue sections were stained by HE. Five fields were randomly selected from each section to count the viable tumor cells, the mean number of viable tumor cells in the endoscopic field was set as N. TCNR = (1 − N/M) × 100%.

2.5. Follow-ups and endpoints

All patients underwent clinical follow-up examinations, the first follow-up time slot was osteosarcoma confirmation, and telephone follow-up was conducted every 3 months thereafter until endpoint events occurs or the end of follow-up. Endpoint events were defined as recurrence, metastasis, or death during the follow-up period, The survival was assessed by the new Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. Overall Survival (OS) was from the day of osteosarcoma confirmation to death or the last day of follow-up. Progression-free Survival (PFS) was the time from the end of previously treated to disease progression such as recurrence or metastasis.

2.6. Statistical analysis

The statistical analyses were conducted with SPSS (version 22.0), the statistical figure was drawn by GraphPad Prism 8.0. Survival curves were generated using the Kaplan–Meier method and compared using the log-rank test. The log- rank test was also used for univariate analyses of prognostic factors. The variables with P-values of < 0.05 from univariate analyses were further analyzed in the multivariate analyses using the Cox proportional hazards regression model. The characteristics of the patients in the two groups were compared using the Chi-square test for categorical variables and the independent sample Student's *t*-test for continuous variables. Tests were two-sided, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. General data

Among 94 cases of osteosarcoma in the study, 61 were male (64.9%) and 33 were female (35.1%);the majority of those aged from 10 to 20 (61.7%);73 patients with BMI < 24 (77.7%), and the rest with BMI ≥ 24 (22.3%); initial diagnosis time of 59 cases were > 30 days (62.8%), and that of 35 cases were ≤ 30 days (37.2%); KPS score of 82 cases were < 90 (66%), but 32 cases were ≥ 90 (34%); the majority of Initial tumor size between 5 and 10 cm (39.4%) (Table 1).

3.2. The expression of TGF-β in osteosarcoma

Because of the storage of pathological specimens, a total of 94 cases were examined for expression of TGF-β in pathological specimens. The pathological results were interpreted by positive cell rate and degree of dyeing (Fig. 1). The positive cell rate scores of 94 patients were 0, 1, 2, 3 and 4 respectively including people of 3 (3.2%), 9 (9.6%), 17 (18%), 15 (16%) and 50 (53.2%). The scores of degree of dyeing were 0, 1, 2 and 3 respectively including people of 3 (3.2%), 10(10.6%), 63(67%) and 18 (19.2%). The product of positive cell rate and degree of dyeing score was taken as the total score, calculating the median of all total scores as 8 as the cutoff value, ≥8 is high expression, < 8 is low expression.45 cases were high expression (47.9%) and 49 cases were low expression (52.1%) (Table 2).

3.2.1. General data in TGF-β high expression group and TGF-β low expression group

94 patients were divided into TGF-β high expression group and TGF-β low expression group According to TGF- β expression, The 12 prognostic factors that may be associated with chemotherapy resistance in previous studies (age, BMI, initial diagnosis time, KPS score, initial tumor size, LWR, NLR, albumin, AST, LDL, BUN, ALP) in the two groups were statistically analyzed. Results show the BMI in TGF-β high expression group was significantly increased compared to TGF-β low expression group ($P = 0.014$), the Initial diagnosis time in TGF-β high expression group was significantly decreased compared to TGF-β low expression group ($P = 0.001$), the KPS in TGF-β high expression group was significantly decreased compared to TGF-β low expression group ($P = 0.033$), the NLR in TGF-β high expression group was significantly increased compared to TGF-β low expression group ($P = 0.029$), the LDL in TGF-β high expression group was significantly increased compared to TGF-β low expression group ($P = 0.039$), the ALP in TGF-β high expression group was significantly increased compared to TGF-β low expression group ($P = 0.007$). There was no statistically significant difference in age, initial tumor size, LWR, albumin, ALT, BUN between the two groups ($P > 0.05$, Fig. 2).

3.2.2. Correlation between TGF-β expression and effect of chemotherapy

After pathological diagnosis of osteosarcoma, 94 osteosarcoma were treated with routine chemotherapy drugs for osteosarcoma (epirubicin/birubicin, cisplatin/nedaplatin, methotrexate, ifosfamide, vincristine), individual differences of patients, the efficacy evaluation was conducted according to the response evaluation criteria in solid tumours by RECIST 1.1. Effect of chemotherapy was positively with positive cell rate ($P < 0.01$ $r = 0.337$) and TGF-β total score ($P < 0.0001$ $r = 0.635$) while effect of chemotherapy was no correlation with degree of dyeing score ($P > 0.05$) (respectively Fig. 3A–C), the higher the

Table 1
Patient baseline characteristics.

Characteristics	n (%)
Gender	
Male	61(64.9)
Female	33(35.1)
Age, years	
< 10	4(4.3)
10–20	58(61.7)
> 20	32(34)
BMI	
< 24	73(77.7)
≥24	21(22.3)
Initial diagnosis time, day	
< 30	59(62.8)
≥30	35(37.2)
KPS scores	
< 90	62(66)
≥90	32(34)
Initial tumor size, cm	
< 5	36(38.3)
5–10	37(39.4)
> 10	21(22.3)

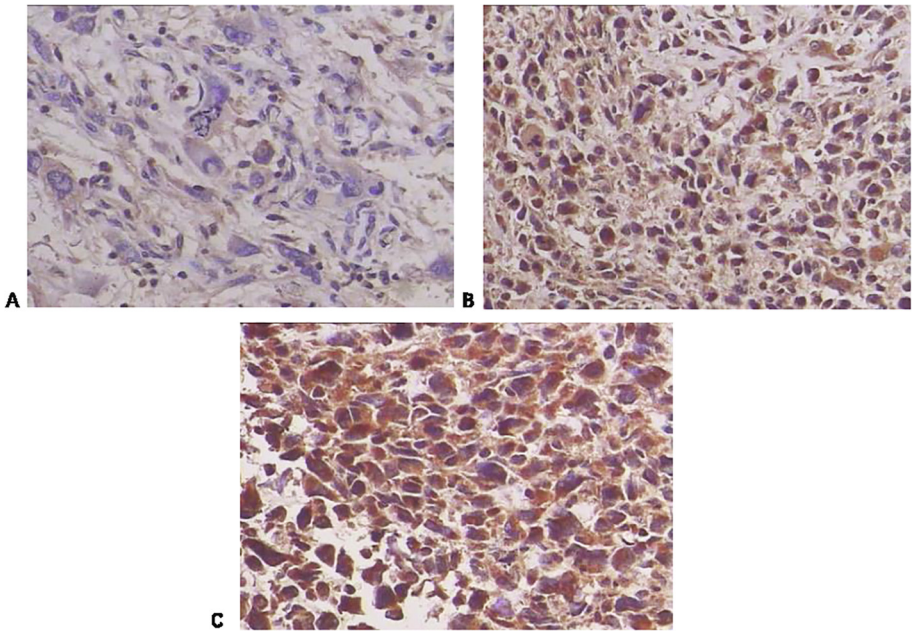

Fig. 1. Pathologic images of TGF-β degree of dyeing: pale yellow is 1 points (A), yellow or brown is 2 points (B), tan is 3 points (C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
TGF-β expression of positive cell rate score, degree of dyeing score and TGF-β total score in osteosarcoma.

Characteristics	All, n (%)
Positive cell rate score	
0	3(3.2)
1	9(9.6)
2	17(18)
3	15(16)
4	50(53.2)
Degree of dyeing score	
0	3(3.2)
1	10(10.6)
2	63(67)
3	18(19.2)
Groups	
TGF-β low expression (< 8)	49(52.1)
TGF-β high expression (≥8)	45(47.9)

TGF-β expression, the higher the change from baseline, which means chemotherapy resistance. There was significant difference in change from baseline after chemotherapy between TGF-β high expression group and TGF-β low expression group (P = 0.045) (Fig. 3D).

3.2.3. Relationship between TGF-β expression and survival prognosis in osteosarcoma

Immunohistochemical TGF-lesion expression was detected in 94 patients and evaluated by pathologists, 45 cases were TGF-β high expression and 49 cases were TGF-β low expression, drawing a survival curve for the two groups of OS, Median OS 61.4 months in the TGF-β high expression group, one-year survival rate, three-year survival rate and five-year survival rate were 82.9%, 58.5% and 51.2% respectively. Median OS 68.1 months in the TGF-β low expression group, one-year survival rate, three-year survival rate and five-year survival rate were 90.2%, 74.1% and 60.5% respectively. There was statistically significant difference in two groups (P = 0.045). Drawing a survival curve for the two groups of PFS, Median PFS 44.8 months in the TGF-β high expression group, Median PFS 56.2 months in the TGF-β low expression group, There was no statistically significant difference in two groups (P > 0.05) (Fig. 4). Results show high expression of TGF-β means poor prognosis.

3.2.4. Expression of TCNR in osteosarcoma after chemotherapy

Because of the storage of pathological specimens, a total of 92 cases were examined for TCNR after chemotherapy. TCNR > 90% is usually considered to be sensitive to chemotherapy. Result show 62 were TCNR ≤ 90% (67.4%), 30 were TCNR > 90% (32.6%) (Fig. 5).

3.3. General data in TCNR ≤ 90% group and TCNR > 90% group

92 patients were divided into TCNR ≤ 90% group and TCNR > 90% group, the 12 prognostic factors that may be associated with chemotherapy resistance in previous studies (age, BMI, initial diagnosis time, KPS score, initial tumor size, LWR, NLR, albumin, AST, LDL, BUN, ALP) in the two groups were statistically analyzed. Results show the initial diagnosis time in TCNR > 90% group was significantly increased compared to TCNR ≤ 90% group (P = 0.014); the KPS in TCNR > 90% group was significantly increased compared to TCNR ≤ 90% group (P = 0.009); the initial tumor size in TCNR > 90% group was significantly decreased compared to TCNR ≤ 90% group (P = 0.007); the BUN in TCNR > 90% group was significantly decreased compared to TCNR ≤ 90% group (P = 0.004); the ALP in TCNR > 90% group was significantly decreased compared to TCNR ≤ 90% group (P = 0.044). There was no statistically significant difference in age, BMI, Lymphocytes/leukocytes, Neutrophils/lymphocytes, albumin, ALT, LDL between the two groups (P > 0.05, Fig. 6).

3.3.1. Correlation between TCNR and effect of chemotherapy

The efficacy evaluation was conducted according to the response evaluation criteria in solid tumours by RECIST 1.1 after chemotherapy. Change from baseline after chemotherapy was negatively with TCNR (P < 0.001 r = −0.411) (Fig. 7A), the smaller the TCNR, the higher the change from baseline, which means chemotherapy resistance, while there was no statistically significant difference between two groups in change from baseline after chemotherapy (P > 0.05) (Fig. 7B).

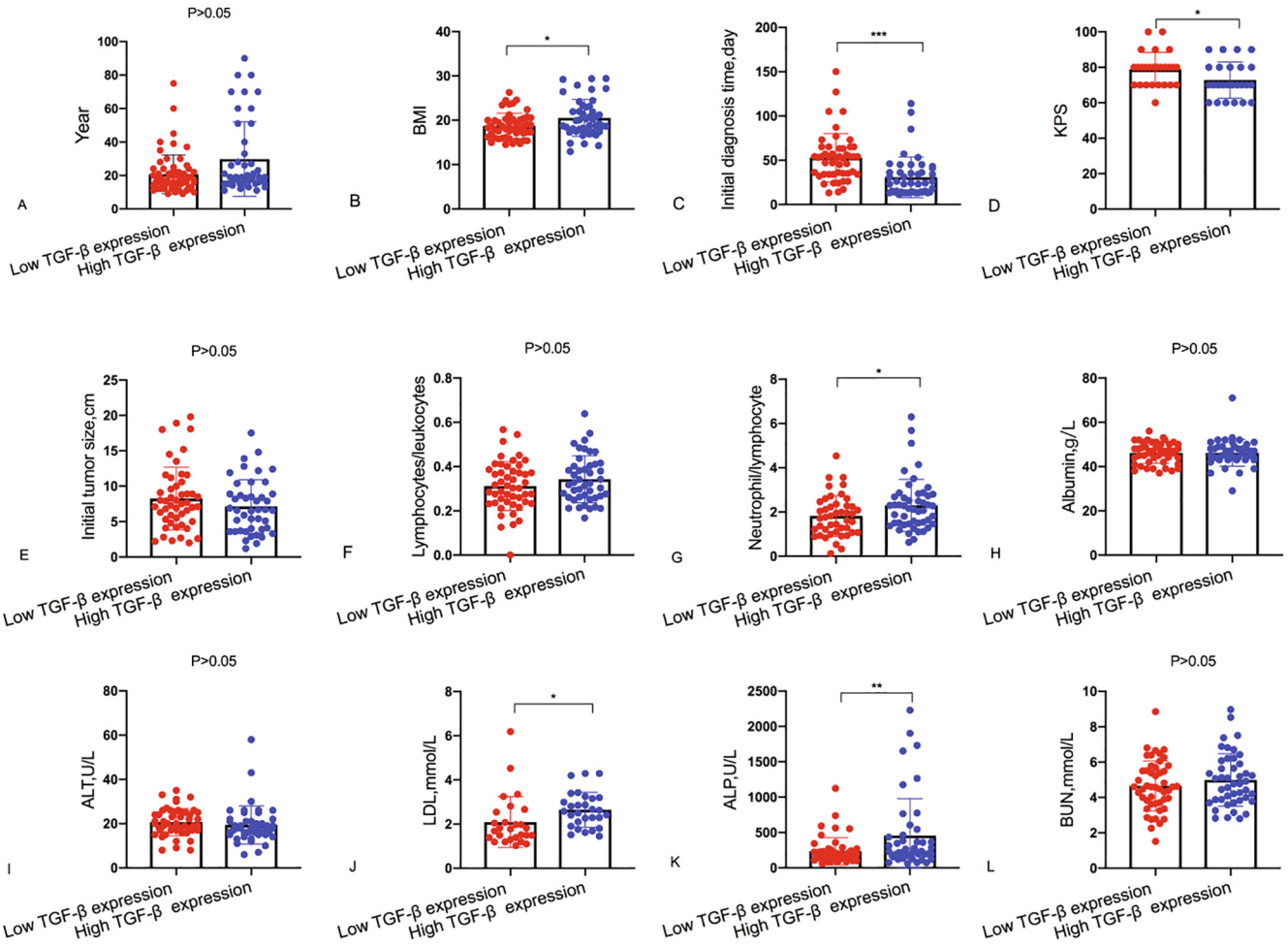

Fig. 2. General data in TGF-β high expression group and TGF-β low expression group: BMI (B), NLR (G), LDL (J), ALP (K) in TGF-β high expression group was significantly increased compared to TGF-β low expression group; Initial diagnosis time (C), KPS (D) in TGF-β high expression group was significantly decreased compared to TGF-β low expression group. (*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.)

3.4. Relationship between TCNR and survival prognosis in osteosarcoma

The tumor cell necrosis rate was detected in 92 cases, 30 cases were TCNR > 90% and 62 cases were TCNR ≤ 90%, drawing a survival curve for the two groups of OS, Median OS 67.8 months in the TCNR > 90% group, one-year survival rate, three-year survival rate and five-year survival rate were 89.3%, 74.1% and 62.1% respectively. Median OS 61.7 months in the TCNR ≤ 90% group, one-year survival rate, three-year survival rate and five-year survival rate were 80.1%, 60.6% and 54.1% respectively, there was statistically significant difference between two groups (P = 0.040). Drawing a survival curve for the two groups of PFS, median PFS 57.4 months in the TCNR > 90% group, median PFS 40.5 months in the TCNR ≤ 90% group, there was statistically significant difference between two groups (P = 0.036) (Fig. 8). Results show TCNR ≤ 90% means poor prognosis.

3.5. Correlation between TGF-β and TCNR

The results show TGF-β score was negatively correlated with TCNR (P < 0.001, r = −0.571) (Fig. 9), which means that the higher the TGF-β score, the lower the TCNR, and this study results suggest that both high TGF-β score and low TCNR maybe mean osteosarcoma chemotherapy drug resistance.

4. Discussion

At present, patients with Osteosarcoma were usually treated by multi-drug neoadjuvant chemotherapy combined with surgery to remove the primary tumor and follow-up adjuvant chemotherapy. The introduction of chemotherapy could significantly improve the average 5-year survival rate of patients with localized diseases [11]. Neoadjuvant chemotherapy can not only prevent micrometastasis in early stage, reducing the risk of distant metastasis, but also being helpful to obtain a negative margin during surgical resection. Bielack reported that response to chemotherapy is a important prognostic factors [11,12]. Conventional neoadjuvant chemotherapy regimen for osteosarcoma is based on a combination of highly cytotoxic drugs such as cisplatin, methotrexate, and doxorubicin [13]. However, acquired drug resistance during the course of treatment reduces the survival rate of patients and has become an important obstacle to survival. To date, many possible mechanisms of resistance to antitumor drugs, especially cisplatin, have been identified, including reduced uptake of cisplatin, increased DNA repair, apoptosis and depolymerization of cisplatin. Although these chemotherapeutic drugs are effective, they are not specific and are resistant to cancer, so the disease Progression and death after chemotherapy are often observed. The cause of develop drug resistance leading to the failure of treatment is mainly due to tumor heterogeneity. Tumor heterogeneity refers to the inconsistency of phenotypes caused by the presence of cells with different genotypes in the same tumor, which is the main reason for the obvious difference in efficacy and drug

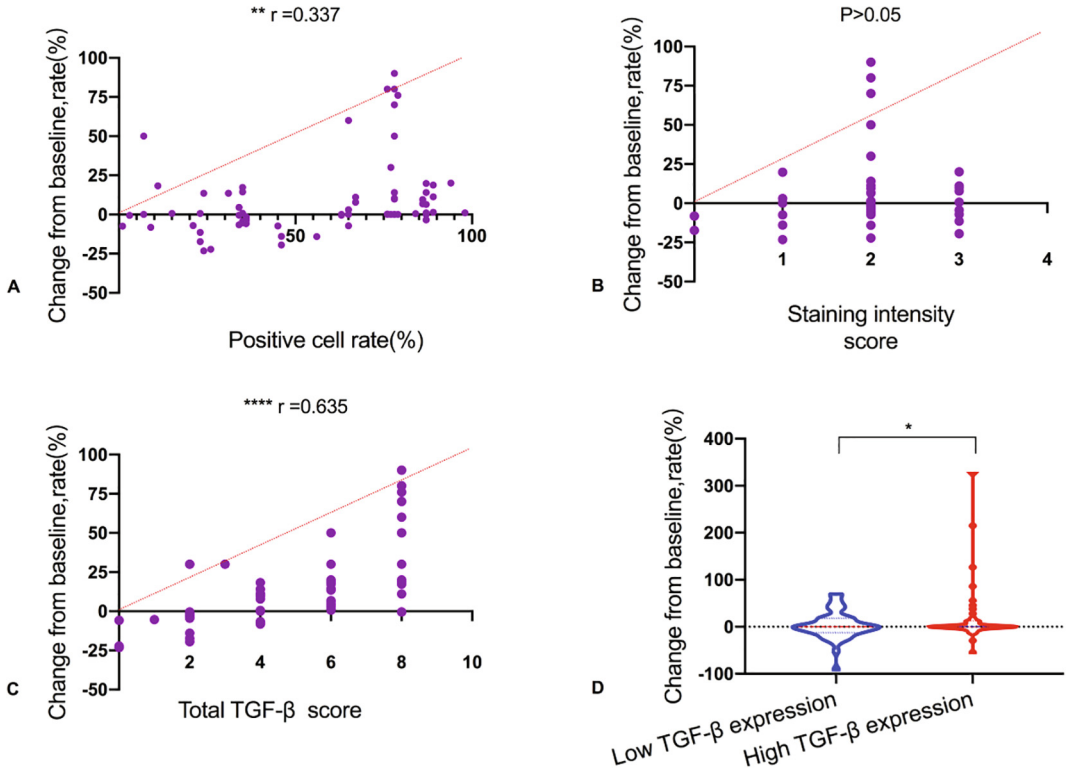

Fig. 3. Correlation between effect of chemotherapy and TGF-β positive cell rate (A), degree of dyeing score (B), TGF-β total score (C); the differences of change from baseline after chemotherapy between TGF-β high expression group and TGF-β low expression group (D) (*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001).

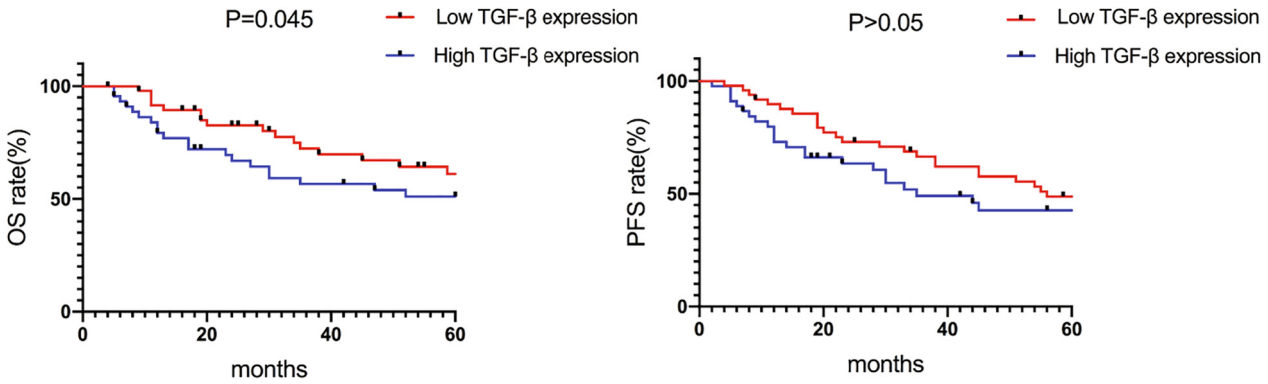

Fig. 4. Relationship between TGF-β expression and survival prognosis in osteosarcoma: OS in TGF-β high expression group was significantly decreased compared to TGF-β low expression group (P = 0.045) (A). PFS was no statistically significant difference in two groups (P > 0.05) (B).

resistance in the process of tumor treatment [14]. In chemotherapy, targeted therapy and other anti-tumor therapies, tumor heterogeneity and tissue differentiation are still the biggest obstacles. Many immunohistochemical studies have attempted to be used as biomarkers for predicting tumor heterogeneity and chemotherapy resistance in osteosarcoma, and TGF-β immunohistochemical and TCNR are considered as predictors of chemotherapy response [15]. Analyzing the expression of markers in surviving tumor tissues after chemotherapy may be similar to assessing the degree of response to chemotherapy and may reflect the characteristics of drug-resistant patients.

The TGF-β superfamily is involved in virtually every aspect of cellular activity, which also can enhance the invasion and metastasis of the tumor by promoting epithelial-mesenchymal transition (EMT) [16]. Lamora et al found TGF-β levels are higher in serum samples from patients with osteosarcoma compared with healthy volunteers [17]. Mohseny et al. found immunohistochemical analysis of phosphorylated Smad2, the intracellular effectors of TGF-β, cases with lower expression showed significantly worse disease free survival. This may imply that drugs restoring impaired signalling pathways in osteosarcoma might change the tumour's aggressive clinical course [18]. The efficacy evaluation was conducted according to the response evaluation criteria in solid tumours by RECIST 1.1. Effect of chemotherapy was positively with positive cell rate (P < 0.01) and TGF-β total score (P < 0.0001), There was significant difference in change from baseline after chemotherapy between TGF-β high expression group and TGF-β low expression group (P < 0.05).

Current research is focused on how to find the risk factors for chemotherapy resistance, there are many methods to evaluate the efficacy of chemotherapy, including clinical symptoms and signs, imaging techniques, laboratory tests and histopathology, the determination of TCNR is considered to be a good method. Anninga et al reported that effective preoperative chemotherapy could cause necrosis of most tumor cells in primary and satellite foci, which was benefit to the tumor excision and the recrudescence rate reduction [19], TCNR is a gold

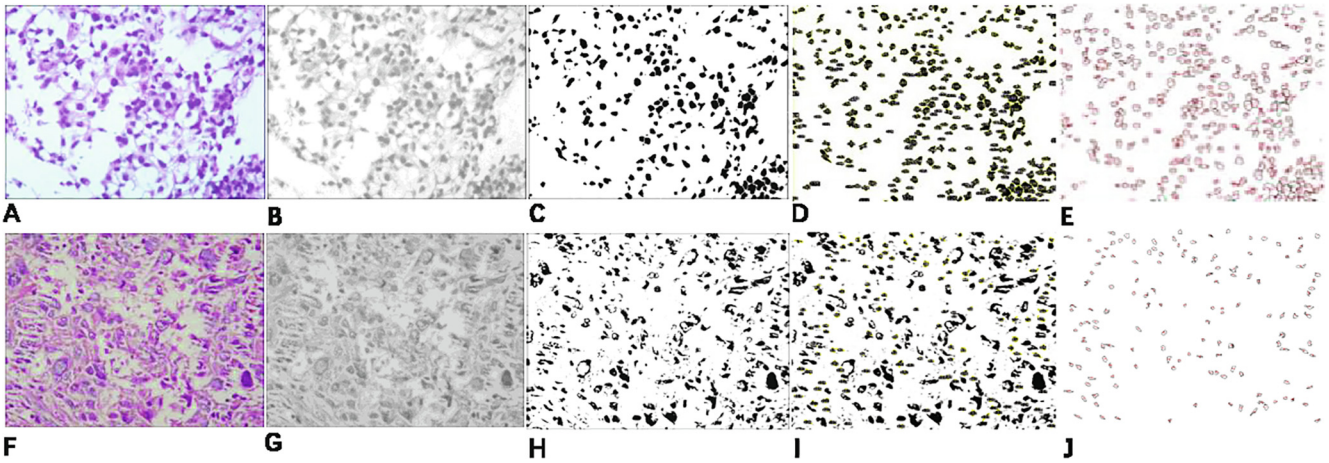

Fig. 5. Counting cells by Image J. A-E: Before chemotherapy; F-J: After chemotherapy. (A, F: H&E × 100): the death of tumor cells increased significantly after chemotherapy.

standard to evaluate the sensitivity to chemotherapy, TCNR > 90% is usually considered to be sensitive to chemotherapy. Goorin et al reported that the preoperative chemotherapy given in this trial was quite effective. Most patients had favorable chemotherapy effects in the primary tumor; 63% of patients had less than 10% residual viable tumor [20]. This study show OS was statistically significant difference between TCNR > 90% group and TCNR ≤ 90% group ($P < 0.05$). Patients whom sensitive to chemotherapy had a longer overall survival. As two important indexes to predict the drug resistance of osteosarcoma, correlation between TGF-β and TCNR show TGF-β score was negatively correlated with TCNR ($P < 0.05$).

During chemotherapy, some tumors mutate themselves to avoid death, TGF-β and TCNR are most important indicators to reflect the sensitivity to chemotherapy which plays an irreplaceable role in the

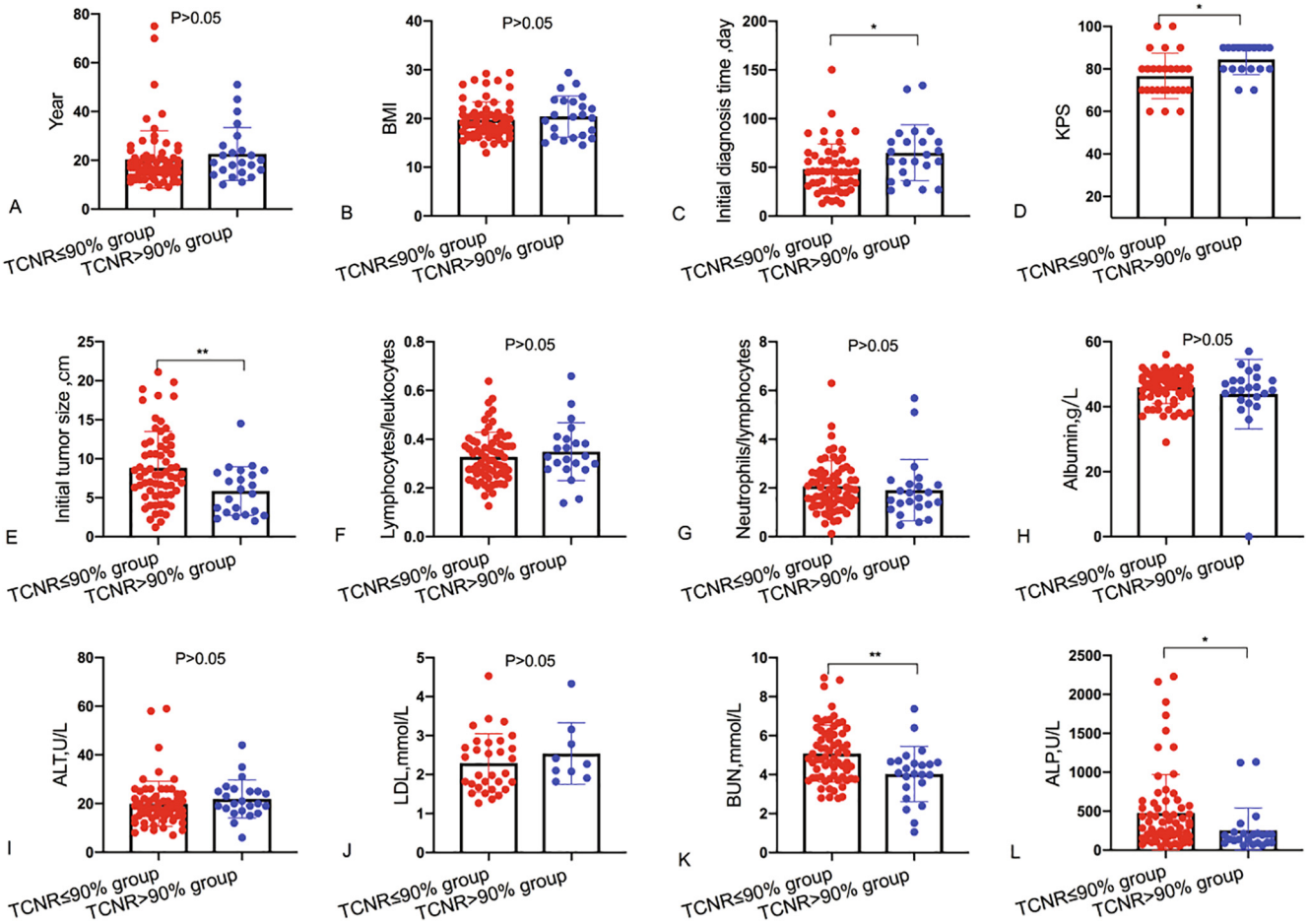

Fig. 6. General data in TCNR ≤ 90% group and TCNR > 90% group: initial diagnosis time (C), KPS (D) in TCNR > 90% group was significantly increased compared to TCNR ≤ 90% group; initial tumor size (E), BUN (K), ALP (L) in TCNR > 90% group was significantly decreased compared to TCNR ≤ 90% group. (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.)

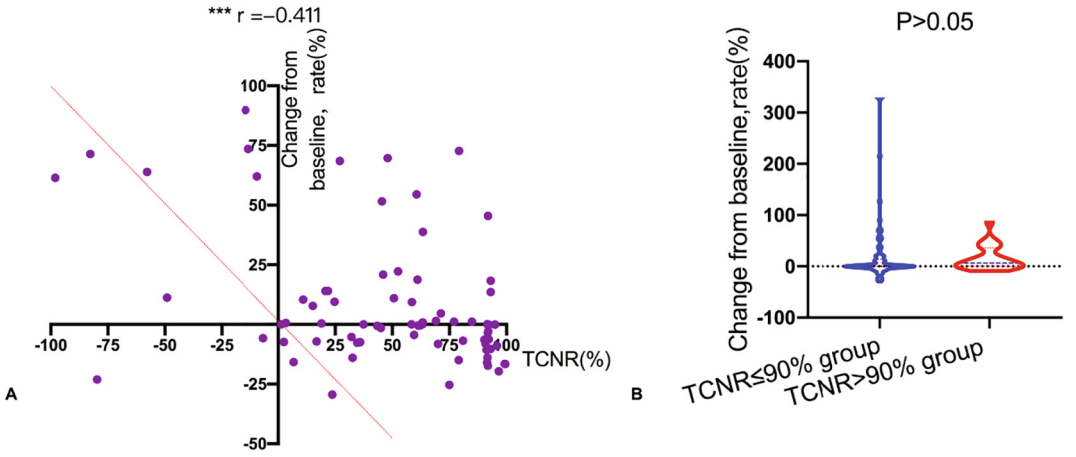

Fig. 7. Correlation between TCNR and effect of chemotherapy: Correlation analysis (A), difference between two groups in change from baseline after chemotherapy (B). (*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.)

evaluation of chemotherapy resistance of osteosarcoma. Tactors effect chemotherapy such as obesity has reached epidemic [21,22]. More than 2.1 billion people are currently overweight or obese, obesity has long been linked to an increased risk of several chronic diseases, but it was only in the last decade that link between obesity and the risk and prognosis of multiple cancers emerged [23]. The underlying mechanism of increased cancer risk in obese patients is associated with multiple molecular and metabolic changes associated with adipose tissue expansion [24], changes in the general and local environment due to obesity not only increase the likelihood of tumor development and progression [25,26], but also have adverse reactions to chemotherapy regimens, leading to resistance to chemotherapy [27,28]. This is consistent with the finding that patients with high TGF-β expression in osteosarcoma more obese than those with low TGF-β expression. In addition, LDL and ALP levels were significantly higher in patients with TGF-β high expression than in TGF-β low expression (P < 0.05), LDL is a normal component of plasma that functions as a transporter of cholesterol in the body, further studies have shown that LDL in some types of cancer is absorbed by tumor cells at a higher rate than normal cells, so the higher the LDL, the more nutrient supply to the tumor cells, worse response to chemotherapy. ALP can accurately reflect the viability of osteoblasts and significantly increased in osteosarcoma, ALP has been proved to be an independent prognostic factor in osteosarcoma [29], In addition, other studies have found that high levels of ALP may be a risk factor for poor prognosis in patients with osteosarcoma after chemotherapy [30], In this study, the level of ALP in TGF-β high expression Group, which represents the drug resistance of osteosarcoma, was significantly increased and ALP level in TCNR > 90%

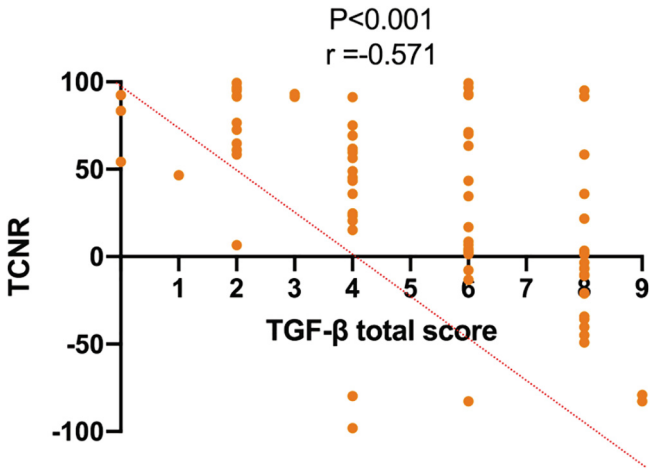

Fig. 9. TGF-β score was negatively correlated with TCNR (P < 0.001, r = −0.571).

group was significantly lower than that in TCNR ≤ 90% group (P < 0.05). The results showed the higher expression of TGF-β, the worse response to chemotherapy, and the easier resistance to chemotherapy.

Immune response has been proved to play a key role in tumor progression, conventional markers of clinical inflammation, such as C-reactive protein and neutrophil, lymphocyte and platelet, expressed individually or in ratios, may provide prognostic information for a

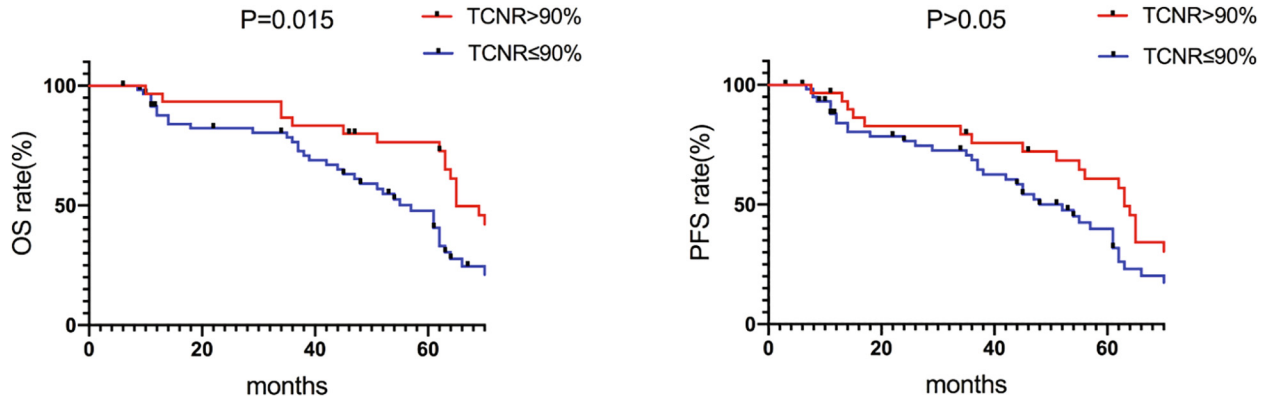

Fig. 8. Relationship between TCNR and survival prognosis in osteosarcoma: OS in TCNR ≤ 90% group was significantly decreased compared to TCNR > 90% group (P = 0.040) (A). PFS was statistically significant difference in two groups (P = 0.036) (B).

variety of cancers, The relationship between NLR and the prognosis of esophageal carcinoma has been clarified, Abe et al found that low LWR was an independent risk factor for OS in a colorectal cancer study[31], However, the study of these markers in osteosarcoma is relatively rare, 94 cases of osteosarcoma study showed NLR in patients with TGF-β high expression was significantly higher than that in patients with TGF-β low expression ($P < 0.05$). NLR has been reported to be associated with clinicopathologic features and survival in a variety of cancers [31,32], NLR had been reported as an independent risk factor for survival in patients with pancreatic cancer in numerous studies and meta-analyses [33,34], this study confirmed that NLR was also closely related to chemotherapy resistance of osteosarcoma. KPS score is an existing tumor performance status standard score, generally used to predict chemotherapy toxicity and survival in all adult cancer patients, the higher KPS score, the better the health, the more likely patient to tolerate the side effects of treatment [35], patients with KPS ≥ 90 are generally considered to be resistant to chemotherapy, this study show KPS scores with high TGF-β expression were lower than those with low TGF-β expression, the KPS score of TCNR > 90% group was significantly higher than that of TCNR ≤ 90% group ($P < 0.05$). The result might be that TGF-β high expression of this part of patients with high tumor cell activity, rapid growth and proliferation, resulting in osteosarcoma patients cachexia state, therefore, the higher the KPS, the lower the TGF-β expression and the better the chemotherapy response. KPS might reflect the expression of risk prognostic factors in patients with osteosarcoma, and thus predict the efficacy and prognosis of chemotherapy.

The initial diagnosis time is the time from symptom onset to first medical attention, it is generally accepted that the time of initial diagnosis time can be used as a criterion for evaluating the degree of disease, patients with severe disease and rapid progress usually have a short initial diagnosis time, while those with mild disease and slow progress have a long initial diagnosis time. This study show the patients with high TGF-β expression had shorter initial diagnosis time than those with low TGF-β expression, The initial diagnosis time of TCNR 90% group was longer than that of TCNR ≤ 90% group. Therefore, the evaluation of the progress of patients admitted to the hospital that might reflect the expression of drug-resistant tumor marker in osteosarcoma to some extent, so as to predict the efficacy of chemotherapy in osteosarcoma and provide sufficient theoretical basis for formulating individualized diagnosis and treatment. Glomerular filtration, tubular secretion, and renal drug metabolism are the processes by which many drugs are cleaned by the kidney, it is clear that impairment of kidney function affects all of these processes, thus affecting kidney clearance of drugs and toxins, which is consistent with the findings of this study, this study result showed BUN in TCNR > 90% group was significantly decreased compared to TCNR ≤ 90% group ($P < 0.05$). The increase of Bun often indicates poor renal function, which leads to the dysfunction of drug metabolism in the kidney, as well as the failure of chemotherapeutic drugs in the body to play the best effect, resulting in chemotherapy resistance, chemotherapy is also a common cause of kidney damage. After kidney damage caused by chemotherapy, the chemotherapy drugs have to be reduced or stopped, and the tumor cells can't be well controlled because they can increase in value more quickly, forming a vicious circle that ultimately killed the patient. Therefore, the evaluation of Renal function in Osteosarcoma chemotherapy effect plays an important role. Initial tumor size is one of the determinants of American Joint Committee on cancer (AJCC) staging and a prognostic factor for patients with osteosarcoma [34], researchers showed the response of the larger lesions to chemotherapy was worse than that of the smaller Lesions [36], which was consistent with the results of this study [37], This study showed initial tumor size in TCNR > 90% group was significantly smaller than that of TCNR ≤ 90% group ($P < 0.05$). Initial tumor-related variables, such as tumor size, location, histological subtype, and biological characteristics, were potential factors in identifying high-risk patients, information about the Initial tumor size is readily available, which is important for developing individualized protocols for patients with large Initial tumor size before chemotherapy [38,39].

This study investigated the expression of TGF-β and TCNR in osteosarcoma and the effect of TGF-β and TCNR on chemotherapy resistance of osteosarcoma, some high-incidence tumors had been well-established in large-scale multicenter studies, but in rare tumors such as Osteosarcoma, all available tissue should be fully utilized for analysis, to obtain more information about the molecular changes of osteosarcoma during chemotherapy, the aim was to obtain valuable information about the survival prognosis or chemotherapy response of patients.

5. Conclusion

The higher the expression of TGF-β, the lower the rate of tumor necrosis, and the more likely the patients with osteosarcoma are to develop chemotherapy resistance and lead to poor prognosis, its expression is of great significance in osteosarcoma, both of which can be served as the basis of individualized treatment for patients with osteosarcoma.

CRediT authorship contribution statement

Ling Zhou: . **Jiadai Tang:** . **Fengdi Hu:** . **Yedan Liao:** . **Rong Li:** . **Yonghong Zhou:** . **Zhihong Yao:** . **Zhengqin Geng:** . **Zuozhang Yang:** . **Xueqi Zhang:** Data curation. **Lin Xie:** .

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 81760520)

Conflict of interest

The authors report no conflicts of interest in this work.

References

[1] B. Abou Ali, M. Salman, K.M. Ghanem, F. Boulos, R. Haidar, S. Saghieh, S. Akel, S.A. Muwakkit, H. El-Solh, R. Saab, H. Tamim, M.R. Abboud, Clinical prognostic factors and outcome in pediatric osteosarcoma: effect of delay in local control and degree of necrosis in a multidisciplinary setting in Lebanon, J. Glob. Oncol. 5 (2019) 1–8, https://doi.org/10.1200/JGO.17.00241.

[2] L. Keller, K. Pantel, Unravelling tumour heterogeneity by single-cell profiling of circulating tumour cells, Nat. Rev. Cancer 19 (10) (2019) 553–567, https://doi.org/10.1038/s41568-019-0180-2.

[3] M. Uhl, U. Saueressig, M. van Buiren, U. Kontny, C. Niemeyer, G. Kohler, K. Ilyasov, M. Langer, Osteosarcoma: preliminary results of in vivo assessment of tumor necrosis after chemotherapy with diffusion- and perfusion-weighted magnetic resonance imaging, Invest. Radiol. 41 (8) (2006) 618–623, https://doi.org/10.1097/01.rli.0000225398.17315.68.

[4] M.A. Huber, N. Kraut, H. Beug, Molecular requirements for epithelial-mesenchymal transition during tumor progression, Curr. Opin. Cell Biol. 17 (5) (2005) 548–558, https://doi.org/10.1016/j.ceb.2005.08.001.

[5] B. Yao, J. Zhao, Y. Li, H. Li, Z. Hu, P. Pan, Y. Zhang, E. Du, R. Liu, Y. Xu, Elf5 inhibits TGF-beta-driven epithelial-mesenchymal transition in prostate cancer by repressing SMAD3 activation, Prostate 75 (8) (2015) 872–882, https://doi.org/10.1002/pros.22970.

[6] T. Brabletz, F. Hlubek, S. Spaderna, O. Schmalhofer, E. Hiendlmeyer, A. Jung, T. Kirchner, Invasion and metastasis in colorectal cancer: epithelial-mesenchymal transition, mesenchymal-epithelial transition, stem cells and beta-catenin, Cells Tissues Organs 179 (1–2) (2005) 56–65, https://doi.org/10.1159/000084509.

[7] Z. Lin, D. Song, H. Wei, X. Yang, T. Liu, W. Yan, J. Xiao, TGF-beta1-induced miR-202 mediates drug resistance by inhibiting apoptosis in human osteosarcoma, J. Cancer Res. Clin. Oncol. 142 (1) (2016) 239–246, https://doi.org/10.1007/s00432-015-2028-9.

[8] D. Brunen, S.M. Willems, U. Kellner, R. Midgley, I. Simon, R. Bernards, TGF-beta: an emerging player in drug resistance, Cell Cycle 12 (18) (2013) 2960–2968, https://doi.org/10.4161/cc.26034.

[9] F. Ji, R. Lv, T. Zhao, A correlation analysis between tumor imaging changes and p-AKT and HSP70 expression in tumor cells after osteosarcoma chemotherapy, Oncol. Lett. 14 (6) (2017) 6749–6753, https://doi.org/10.3892/ol.2017.7005.

[10] J.M.M. Cates, Simple staging system for osteosarcoma performs equivalently to the

AJCC and MSTS systems, J. Orthop. Res. 36 (10) (2018) 2802–2808, https://doi.org/10.1002/jor.24032.
[11] S. Rello, J.C. Stockert, V. Moreno, A. Gamez, M. Pacheco, A. Juarranz, M. Canete, A. Villanueva, Morphological criteria to distinguish cell death induced by apoptotic and necrotic treatments, Apoptosis 10 (1) (2005) 201–208, https://doi.org/10.1007/s10495-005-6075-6.
[12] S.S. Bielack, B. Kempf-Bielack, G. Delling, G.U. Exner, S. Flege, K. Helmke, R. Kotz, M. Salzer-Kuntschik, M. Werner, W. Winkelmann, A. Zoubek, H. Jurgens, K. Winkler, Prognostic factors in high-grade osteosarcoma of the extremities or trunk: an analysis of 1,702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols, J. Clin. Oncol. 20 (3) (2002) 776–790, https://doi.org/10.1200/JCO.2002.20.3.776.
[13] P.C. Hogendoorn, E.E.W. Group, N. Athanasou, S. Bielack, E. De Alava, A.P. Dei Tos, S. Ferrari, H. Gelderblom, R. Grimer, K.S. Hall, B. Hassan, P.C. Hogendoorn, H. Jurgens, M. Paulussen, L. Rozeman, A.H. Taminiau, J. Whelan, D. Vanel, Bone sarcomas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, Ann. Oncol. 21 (Suppl 5) (2010) v204–v213, https://doi.org/10.1093/annonc/mdq223.
[14] P.L. Bedard, A.R. Hansen, M.J. Ratain, L.L. Siu, Tumour heterogeneity in the clinic, Nature 501 (7467) (2013) 355–364, https://doi.org/10.1038/nature12627.
[15] V. Tripathi, J.H. Shin, C.H. Stuelten, Y.E. Zhang, TGF-beta-induced alternative splicing of TAK1 promotes EMT and drug resistance, Oncogene 38 (17) (2019) 3185–3200, https://doi.org/10.1038/s41388-018-0655-8.
[16] R. Yang, S. Piperdi, Y. Zhang, Z. Zhu, N. Neophytou, B.H. Hoang, G. Mason, D. Geller, H. Dorfman, P.A. Meyers, J.H. Healey, D.G. Phinney, R. Gorlick, Transcriptional profiling identifies the signaling axes of IGF and transforming growth factor-b as involved in the pathogenesis of osteosarcoma, Clin. Orthop. Relat. Res. 474 (1) (2016) 178–189, https://doi.org/10.1007/s11999-015-4578-1.
[17] A. Lamora, J. Talbot, G. Bougras, J. Amiaud, M. Leduc, J. Chesneau, J. Taurelle, V. Stresing, M.C. Le Deley, M.F. Heymann, D. Heymann, F. Redini, F. Verrecchia, Overexpression of smad7 blocks primary tumor growth and lung metastasis development in osteosarcoma, Clin. Cancer Res. 20 (19) (2014) 5097–5112, https://doi.org/10.1158/1078-0432.CCR-13-3191.
[18] A.B. Mohseny, Y. Cai, M. Kuijjer, W. Xiao, B. van den Akker, C.E. de Andrea, R. Jacobs, P. ten Dijke, P.C. Hogendoorn, A.M. Cleton-Jansen, The activities of Smad and Gli mediated signalling pathways in high-grade conventional osteosarcoma, Eur. J. Cancer 48 (18) (2012) 3429–3438, https://doi.org/10.1016/j.ejca.2012.06.018.
[19] J.K. Anninga, H. Gelderblom, M. Fiocco, J.R. Kroep, A.H. Taminiau, P.C. Hogendoorn, R.M. Egeler, Chemotherapeutic adjuvant treatment for osteosarcoma: where do we stand? Eur. J. Cancer 47 (16) (2011) 2431–2445, https://doi.org/10.1016/j.ejca.2011.05.030.
[20] A.M. Goorin, D.J. Schwartzentruber, M. Devidas, M.C. Gebhardt, A.G. Ayala, M.B. Harris, L.J. Helman, H.E. Grier, M.P. Link, G. Pediatric Oncology, Presurgical chemotherapy compared with immediate surgery and adjuvant chemotherapy for nonmetastatic osteosarcoma: Pediatric Oncology Group Study POG-8651, J. Clin. Oncol. 21 (8) (2003) 1574–1580, https://doi.org/10.1200/JCO.2003.08.165.
[21] K.M. Flegal, M.D. Carroll, B.K. Kit, C.L. Ogden, Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999–2010, JAMA 307 (5) (2012) 491–497, https://doi.org/10.1001/jama.2012.39.
[22] P. Hossain, B. Kawar, M. El Nahas, Obesity and diabetes in the developing world–a growing challenge, N. Engl. J. Med. 356 (3) (2007) 213–215, https://doi.org/10.1056/NEJMp068177.
[23] E.E. Calle, C. Rodriguez, K. Walker-Thurmond, M.J. Thun, Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults, N. Engl. J. Med. 348 (17) (2003) 1625–1638, https://doi.org/10.1056/NEJMoa021423.
[24] A.E. Harvey, L.M. Lashinger, G. Otto, N.P. Nunez, S.D. Hursting, Decreased systemic IGF-1 in response to calorie restriction modulates murine tumor cell growth, nuclear factor-kappaB activation, and inflammation-related gene expression, Mol. Carcinog. 52 (12) (2013) 997–1006, https://doi.org/10.1002/mc.21940.
[25] R.E. De Angel, J.M. Blando, M.G. Hogan, M.A. Sandoval, P.D. Lansakara, S.M. Dunlap, S.D. Hursting, Z. Cui, Stearoyl gemcitabine nanoparticles overcome obesity-induced cancer cell resistance to gemcitabine in a mouse postmenopausal breast cancer model, Cancer Biol. Ther. 14 (4) (2013) 357–364, https://doi.org/10.4161/cbt.23623.
[26] L.M. Nogueira, S.M. Dunlap, N.A. Ford, S.D. Hursting, Calorie restriction and rapamycin inhibit MMTV-Wnt-1 mammary tumor growth in a mouse model of postmenopausal obesity, Endocr. Relat. Cancer 19 (1) (2012) 57–68, https://doi.org/10.1530/ERC-11-0213.
[27] C.J. Fabian, B.F. Kimler, J.E. Donnelly, D.K. Sullivan, J.R. Klemp, B.K. Petroff, T.A. Phillips, T. Metheny, S. Aversman, H.W. Yeh, C.M. Zalles, G.B. Mills, S.D. Hursting, Favorable modulation of benign breast tissue and serum risk biomarkers is associated with > 10% weight loss in postmenopausal women, Breast Cancer Res. Treat. 142 (1) (2013) 119–132, https://doi.org/10.1007/s10549-013-2730-8.
[28] L.M. Lashinger, E.L. Rossi, S.D. Hursting, Obesity and resistance to cancer chemotherapy: interacting roles of inflammation and metabolic dysregulation, Clin. Pharmacol. Ther. 96 (4) (2014) 458–463, https://doi.org/10.1038/clpt.2014.136.
[29] X. Nie, D. Liu, Q. Li, C. Bai, Predicting chemotherapy toxicity in older adults with lung cancer, J. Geriatr. Oncol. 4 (4) (2013) 334–339, https://doi.org/10.1016/j.jgo.2013.05.002.
[30] R. Gu, Y. Sun, Does serum alkaline phosphatase level really indicate the prognosis in patients with osteosarcoma? A meta-analysis, J. Cancer Res. Ther. 14 (Suppl.) (2018) S468–S472, https://doi.org/10.4103/0973-1482.177217.
[31] H. Hao, L. Chen, D. Huang, J. Ge, Y. Qiu, L. Hao, Meta-analysis of alkaline phosphatase and prognosis for osteosarcoma, Eur. J. Cancer Care (Engl.) 26 (5) (2017), https://doi.org/10.1111/ecc.12536.
[32] G.J. Guthrie, K.A. Charles, C.S. Roxburgh, P.G. Horgan, D.C. McMillan, S.J. Clarke, The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer, Crit. Rev. Oncol. Hematol. 88 (1) (2013) 218–230, https://doi.org/10.1016/j.critrevonc.2013.03.010.
[33] S. Abe, K. Kawai, H. Nozawa, K. Hata, T. Kiyomatsu, T. Morikawa, T. Watanabe, LMR predicts outcome in patients after preoperative chemoradiotherapy for stage II-III rectal cancer, J. Surg. Res. 222 (2018) 122–131, https://doi.org/10.1016/j.jss.2017.09.053.
[34] Q. Ben, W. An, L. Wang, W. Wang, L. Yu, Y. Yuan, Validation of the pretreatment neutrophil-lymphocyte ratio as a predictor of overall survival in a cohort of patients with pancreatic ductal adenocarcinoma, Pancreas 44 (3) (2015) 471–477, https://doi.org/10.1097/MPA.0000000000000271.
[35] M. Stotz, A. Gerger, F. Eisner, J. Szkandera, H. Loibner, A.L. Ress, P. Kornprat, W. AlZoughbi, F.S. Seggewies, C. Lackner, T. Stojakovic, H. Samonigg, G. Hoefler, M. Pichler, Increased neutrophil-lymphocyte ratio is a poor prognostic factor in patients with primary operable and inoperable pancreatic cancer, Br. J. Cancer 109 (2) (2013) 416–421, https://doi.org/10.1038/bjc.2013.332.
[36] H. Cheng, F. Long, M. Jaiswar, L. Yang, C. Wang, Z. Zhou, Prognostic role of the neutrophil-to-lymphocyte ratio in pancreatic cancer: a meta-analysis, Sci. Rep. 5 (2015) 11026, https://doi.org/10.1038/srep11026.
[37] T. Shibue, R.A. Weinberg, EMT, CSCs, and drug resistance: the mechanistic link and clinical implications, Nat. Rev. Clin. Oncol. 14 (10) (2017) 611–629, https://doi.org/10.1038/nrclinonc.2017.44.
[38] F.J. Lopez-Diaz, P. Gascard, S.K. Balakrishnan, J. Zhao, S.V. Del Rincon, C. Spruck, T.D. Tlsty, B.M. Emerson, Coordinate transcriptional and translational repression of p53 by TGF-beta1 impairs the stress response, Mol. Cell 50 (4) (2013) 552–564, https://doi.org/10.1016/j.molcel.2013.04.029.
[39] E. Papa, M. Weller, T. Weiss, E. Ventura, I. Burghardt, E. Szabo, Negative control of the HGF/c-MET pathway by TGF-beta: a new look at the regulation of stemness in glioblastoma, Cell Death Dis. 8 (12) (2017) 3210, https://doi.org/10.1038/s41419-017-0051-2.