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Diagnostic Performance of Serum miR-586 and miR-493 in Patients with Osteosarcoma

Hao Dang*

Department of Orthopaedics and Traumatology, The First Affiliated Hospital of Guangxi Medical University, Nanning, China
*Corresponding Author: Hao Dang. Email: Danghao@163.com

Abstract: This study aimed to explore the expression and diagnostic performance of miR-586 and miR-493 in patients with osteosarcoma. We enrolled 63 patients with osteosarcoma treated between May 2016 and May 2019 as the experimental group and 55 healthy volunteers who underwent physical examination during the same period as the control group. We detected miR-586 and miR-493 expression in serum, normal bone tissue, and tumor samples. We also evaluated the relationship between clinical indicators and the miR-586 and miR-493 expression levels. The correlation between the serum and osteosarcoma tissue expression of miR-586 and miR-493 was examined by Pearson analysis. Furthermore, the diagnostic performance of miR-586 and miR-493 in osteosarcoma was determined by the ROC curve analysis. The experimental group had higher serum miR-586 expression levels and lowered serum miR-493 expression levels than the control group ($p < 0.05$ on both). In tumor tissues, the miR-586 expression level was higher, whereas the miR-493 expression level was lower than in normal bone tissues ($p < 0.05$). The serum and tumor tissue expression of miR-586 and miR-493 had a positive correlation ($p < 0.05$). The AUC, cutoff level, sensitivity, and specificity of miR-586 for osteosarcoma were 0.8413, 66.435, 66.67, and 98.18, whereas those of miR-493 were 0.7763, 62.540, 82.53, and 78.18, respectively. Patients with osteosarcoma exhibited up-regulated miR-586 levels and down-regulated miR-493 levels in serum and tumor tissues significantly.

Keywords: Hepatocellular carcinoma; sinomenine; ingenuity pathway analysis

1 Introduction

Osteosarcoma is a neoplasm derived from primitive bone-forming mesenchymes. Teens (aged between 10 and 30 years) are the most commonly affected age group, but people of any age can develop osteosarcoma [1–3]. Despite its rarity, osteosarcoma is still the most common primary malignant tumor of the bone. Annually, around 900 new cases are reported in the American Cancer Society, accounting for 3.4% of the total number of childhood cancers; this percentage ranks osteosarcoma as the fifth most common cancer among adolescents. Regardless of the type of treatment option is administered, the overall survival rate remains at approximately 70%, which demonstrates no improvement within 30 years [4]. Therefore, researchers aimed to obtain an in-depth understanding of osteosarcoma's occurrence and development and explore its related influential molecules.

MicroRNA (miRNA) is a type of endogenous noncoding RNA molecule that may enhance mRNA translation and induce gene expression by binding to the complementary sequence of the target messenger RNA transcript (mRNA) 3′ untranslated region (3′-UTR), which regulates the expression of human protein-encoding genes. To regulate gene expression, the 3′-UTR suppresses gene miRNA translation and reduces miRNA stability; its abnormal regulation is associated with most cancers [5–7]. In particular, miR-586,

which is located on chromosome 11p15.5, is significantly up-regulated in many diseases, such as nephrotic syndrome and acute graft-versus-host disease. Wang et al. revealed that miR-586 is a new biomarker for diagnosing acute graft-versus-host disease [8–11]. miR-493 is significantly decreased in lung cancer and can directly target E2F1, thereby inhibiting the growth, invasion, and metastasis of cancer cells; the same phenomenon was observed in gastric cancer, colon cancer, and melanoma [12–14].

However, the expression level and diagnostic value of these two miRNAs in osteosarcoma are rarely reported. Hence, this study aimed to provide more data of these two miRNAs for osteosarcoma clinical diagnosis.

2 Patients and Methods

2.1 General Information

We selected 63 patients with osteosarcoma treated in our hospital (The first affiliated hospital of Guangxi Medical University, Nanjing, Guangxi, China) from May 2016 to May 2019 as the experimental group and 55 healthy volunteers who underwent physical examination as the control group. Their mean age was 33.24 ± 12.43 years (20–46 years).

2.2 Inclusion and Exclusion Criteria

The inclusion criteria for the experimental group were those who were diagnosed with osteosarcoma by imaging and biopsy in our hospital and who had no autoimmune disease. Healthy controls constituted the control group. Conversely, the exclusion criteria were women experiencing pregnancy and lactation; those with antitumor therapy with preoperative radiotherapy and chemotherapy; those with abnormal bleeding or abnormal blood coagulation; and those with history of other tumor diseases and neurological dysfunction. The ethics committee of the hospital approved this study, and the written informed consent was signed either by patients or their families.

2.3 Observation Indicators

The detection of expression of miR-586 and miR-493 was performed in the normal bone tissues, serum, and osteosarcoma tissues. We also analyzed their relationship with the clinical indicators of patients with osteosarcoma.

2.4 Detection Method

2.4.1 Sample Processing and RNA Extraction

We collected the peripheral venous blood of the subjects (that of the experimental group should be collected before neoadjuvant chemotherapy), followed by centrifugation at 2500 r/min for 10 min at room temperature. The supernatant was stored in 1.5 ml PE tube at −80ºC. Furthermore, the osteosarcoma tissue and its surrounding normal bone tissues were collected and stored in liquid nitrogen. Total RNA was extracted according to the procedures specified for the RNA extraction kit (Merck Sigma-Aldrich). The concentration and purity of the total RNA were measured by ultraviolet spectrophotometry, whereas its integrity was detected by electrophoresis.

2.4.2 qRT-PCR

The total RNA was extracted from serum and cultured cells by using the Trizol extraction kit (Invitrogen, Carlsbed, CA, USA). To detect the RNA concentration and purity, we used the Nano-Drop2000 ultraviolet spectrophotometer (Beijing Keyu Xingye Technology Development Co., Ltd., Beijing, China). Using the Takara reverse transcription kit (Invitrogen, Carlsbed, CA, USA), we reverse-transcribed the RNA into cDNA. The synthesized cDNA was then stored at −20ºC until use. The primers were designed and synthesized by

Shanghai Jima Pharmaceutical Technology Co., Ltd., in China (Shanghai, China). The reactions were performed on the ABI PRISM 7500 fluorescent quantitative PCR instrument (Applied Biosystems, Foster, CA, USA). PCR amplification conditions were as follows: 90ºC for 5 min, 90ºC for 5 s, 60ºC for 30 s, and 72ºC for 5 s conducted over 40 cycles. Each sample was tested thrice, and the relative gene expression was determined by the $2^{-\Delta\Delta CT}$ method.

2.5 Statistical Methods

Data were analyzed by SPSS19.0 (AsiaAnalytics [formerly SPSS China]). The count data represented by [n (%)] was compared by the χ^2 test, whereas the measurement data indicated by $\bar{x} \pm sd$ was compared by independent sample *t*-test. The multiple groups were compared by the analysis of variance. The correlation between the serum and tumor tissue expression of miR-586 and miR-493 was examined by Pearson analysis, and the diagnostic performance of these two miRNAs for osteosarcoma was determined by ROC curve analysis.

3 Results

3.1 Baseline Data

No difference was found between the two groups in terms of gender, BMI, age, tumor diameter, tumor type, tumor, tumor stage, and metastasis ($p < 0.05$, Tab. 1).

Table 1: Baseline data

	Experimental group (n = 63)	Control group (n = 55)	χ^2/t	*p*
Gender [n (%)]			0.025	0.873
Male	33 (52.38)	28 (50.91)		
Female	30 (47.62)	27 (49.09)		
BMI (kg/m ^{2})	23.42 ± 1.31	23.24 ± 1.27	0.755	0.452
Age (year)			0.035	0.851
>20	23 (36.51)	21 (38.18)		
≤20	40 (63.49)	34 (61.82)		
Tumor diameter [n (%)]			-	-
≥8 cm	28 (44.44)	-		
<8 cm	35 (55.56)	-		
Tumor type [n (%)]			-	-
Osteoblast type	25 (39.68)	-		
Chondrocyte type	16 (25.40)	-		
Fibroblast type	12 (19.05)	-		
Mixed type	10 (15.87)	-		
Tumor site [n (%)]			-	-
Femur	43 (68.25)	-		
Tibia and fibula	11 (17.46)	-		
other	9 (14.29)	-		
Clinical stage [n (%)]			-	-
I–IIA	27 (42.86)	-		
IIB–III	36 (57.14)	-		
Metastasis [n (%)]			-	-
Yes	26 (41.27)	-		
No	37 (58.73)	-		

3.2 Detection of the miR-586 and miR-493 Expression Levels

The experimental group showed increased expression level of serum miR-586 and decreased serum miR-493compared with the control group ($p < 0.05$) while showed. Moreover, the tumor tissues exhibited increased miR-586 expression levels and decreased miR-493 expression levels compared with the normal bone tissues surrounding the tumor ($p < 0.05$, Tabs. 2 and 3).

Table 2: Serum miR-586 and miR-493 levels

	Control group (n = 55)	Experimental group (n = 63)	*t*	*p*
miR-586	0.85 ± 0.21	1.45 ± 0.51	8.140	<0.001
miR-493	1.18 ± 0.14	0.62 ± 0.11	24.300	<0.001

Table 3: Expression levels of two miRNAs in normal bone tissues and osteosarcoma tissues

	Normal bone tissue	Osteosarcoma tissue	*t*	*p*
miR-586	0.93 ± 0.35	5.14 ± 0.24	76.990	<0.001
miR-493	1.16 ± 0.25	0.51 ± 0.13	18.040	<0.001

3.3 Correlation between Serum and Tumor Tissue Expression of These miRNAs

The serum levels of miR-586 and miR-493 showed positive correlation with the tumor tissue levels of these miRNAs ($p < 0.05$, Fig. 1).

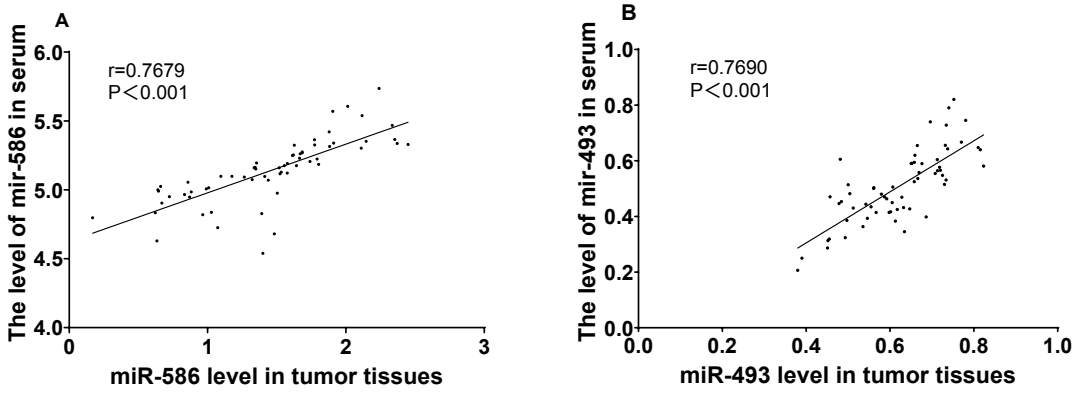

Figure 1: Correlation of the expression of miRNAs between serum and tumor tissues. A: Correlation between serum and tumor tissues in terms of miR-586 expression in patients with osteosarcoma. B: Correlation between serum and tumor tissues in terms of miR-493 expression in patients with osteosarcoma. The miR-586 level in serum positively correlated with that in tumor tissues (r = 0.7679, $p < 0.001$). The miR-493 level in serum positively correlated with that in tumor tissues (r = 0.7690, $p < 0.001$)

3.4 Relationship between the Expression Levels of the Two miRNAs and the Clinical Indicators

The expression of serum miR-586 and miR-493 may be related to tumor size, clinical stage, and metastasis (Tabs. 4 and 5).

Table 4: Relationship between the miR-586 expression level and the clinical indexes of patients with osteosarcoma

	n	miR-586	*t*/F	*p*
Gender [n (%)]			1.540	0.129
Male	33	1.51 ± 0.03		
Female	30	1.50 ± 0.02		
Age (year)			0.819	0.416
>20	23	1.49 ± 0.04		
≤20	40	1.48 ± 0.05		
Tumor diameter [n (%)]			12.470	<0.001
≥8 cm	28	1.57 ± 0.04		
<8 cm	35	1.46 ± 0.03		
Tumor type [n (%)]			1.302	0.282
Osteoblast type	25	1.48 ± 0.02		
Chondrocyte type	16	1.49 ± 0.03		
Fibroblast type	12	1.47 ± 0.02		
Hybrid	10	1.48 ± 0.04		
Tumor site [n (%)]			1.569	0.217
Femur	43	1.47 ± 0.04		
Tibia and fibula	11	1.49 ± 0.03		
other	9	1.46 ± 0.05		
Clinical stage [n (%)]			7.844	<0.001
I–IIA	27	1.45 ± 0.02		
IIB–III	36	1.53 ± 0.05		
Metastasis [n (%)]			6.806	<0.001
Yes	26	1.54 ± 0.04		
No	37	1.48 ± 0.03		

Table 5: Relationship between the miR-493 expression level and the clinical indexes of patients with osteosarcoma

	n	miR-493	*t*/F	*p*
Gender [n (%)]			1.940	0.057
Male	33	0.62 ± 0.07		
Female	30	0.65 ± 0.05		
Age (year)			1.911	0.061
>20	23	0.61 ± 0.04		
≤20	40	0.63 ± 0.04		
Tumor diameter [n (%)]			6.149	<0.001
≥8cm	28	0.57 ± 0.09		
<8cm	35	0.68 ± 0.05		
Tumor type [n (%)]			2.194	0.098
Osteoblast type	25	0.61 ± 0.02		
Chondrocyte type	16	0.63 ± 0.03		
Fibroblast type	12	0.62 ± 0.02		
Mixed type	10	0.62 ± 0.03		
Tumor site [n (%)]			1.569	0.217
Femur	43	0. 62 ± 0.04		

Tibia and fibula	11	0.64 ± 0.03		
other	9	0.61 ± 0.05		
Clinical stage [n (%)]			8.819	<0.001
I–IIA	27	0.69 ± 0.05		
IIB–III	36	0.59 ± 0.04		
Metastasis [n (%)]			10.380	<0.001
Yes	26	0.60 ± 0.02		
No	37	0.67 ± 0.03		

3.5 Diagnostic Performance of Two miRNAs for Osteosarcoma

The AUC, critical level, sensitivity, and specificity of miR-586 for osteosarcoma were 0.8413, 66.435, 66.67, and 98.18, whereas those of miR-493 were 0.7763, 62.540, 82.53, and 78.18, respectively (Tab. 6, Fig. 2).

Table 6: Diagnostic performance of miR-586 and miR-493 for osteosarcoma

	AUC	Cut off	95%Cl	Sensitivity%	Specificity%
miR-586	0.8413	66.435	0.7647 to 0.9178	66.67	98.18
miR-493	0.7763	62.540	0.6786 to 0.8740	82.53	78.18

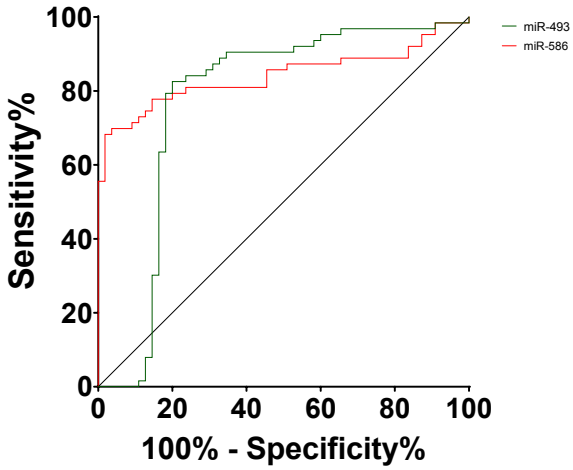

Figure 2: Diagnostic value of miR-586 and miR-493 for osteosarcoma

The AUC values of miR-586 and miR-493 in osteosarcoma diagnosis were 0.8413 and 0.7763, respectively.

4 Discussion

Osteosarcoma is a highly heterogeneous malignant primary tumor of the bone, characterized by vascular invasion and local soft-tissue infiltration [15,16]. The expression level of miRNAs is crucially related to osteosarcoma occurrence. Qian et al. [17] believe that miR-493 is significantly down-regulated in tissues and cell lines; its forced expression significantly reduces the proliferation and invasion abilities of osteosarcoma cells, but through the negative regulation of SP1, such proliferation and invasion abilities are inhibited. Yang et al. [18] found that the down-regulation of miR-586 expression in osteosarcoma can inhibit cell proliferation, invasion, and metastasis and promote apoptosis. Although these studies have confirmed the roles of miR-493 and miR-586 in osteosarcoma, the gathered details are still insufficient.

Present study showed that the experimental group showed increased serum expression level of miR-586 and decreased miR-493 level compared with the control group. Yang [18] revealed that the miR-586 level is significantly increased, while Cao et al. [19] found that the miR-493 level is decreased in osteosarcoma. The up-regulation and/or down-regulation of miRNA expression in cancer indicates that miRNAs as classic tumor suppressor genes or oncogenes may contribute to tumor stratification and clinical prognosis, and the expression of a specific miRNA may be related to tumor recurrence. Therefore, the apparent difference between their expression in normal cells and abnormal cells may be related to the early diagnosis, treatment, or recurrence of primary cancer [20]. To prove that the expression levels of the two miRNAs in serum can also indicate their expression levels in osteosarcoma tissues, we compared the expression levels of these miRNAs between serum and osteosarcoma tissues and found a positive correlation. Hence, the expression levels of these two miRNAs in serum fairly reflect those in the tumor tissue, thereby making the diagnosis simpler.

We also explored the relationship between the serum expression of miR-586 and miR-493 and the clinical indexes. We found that the serum expression levels of miR-586 and miR-493 were not related to gender, age, tumor type, and tumor site but were related to the tumor size; in other words, the larger the tumor, the higher the miR-586, and the lower the miR-493 in the serum. In the clinical staging of osteosarcoma, the higher the stage of malignancy, the higher the miR-586, and the lower the miR-493. This finding is also related to the condition of metastasis; hence, we speculate that the higher the severity of osteosarcoma, the higher the miR-586, and the lower the miR-493. According to Sarver et al. [21], compared with primary osteosarcoma tumors without metastasis, 14q32 miRNAs were more significantly down-regulated after metastasis, suggesting that miR-586 and miR-493 are involved in aneurysm development and metastasis. Therefore, the expression of miR-586 and miR-493 can be utilized to determine disease severity.

Finally, we analyzed the diagnostic performance of these miRNAs in serum for osteosarcoma and found that the areas under the curve were 0.8413 and 0.7763; both of these values had a certain diagnostic value for osteosarcoma. Liu et al. [22] found that miR-586 is highly expressed in osteosarcoma, indicating that it has a potential value for osteosarcoma diagnosis, thereby confirming the results of our study.

However, this study has certain limitations. The molecular mechanisms of miR-586 and miR-493 in osteosarcoma have not been analyzed. Hence, we will explore such mechanisms in future studies.

In summary, the miR-493 expression levels were low, whereas the miR-586 expression levels were high in the serum and tumor tissue of patients with osteosarcoma. The detection of these two miRNAs in serum may help in the clinical diagnosis of osteosarcoma.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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