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RESEARCH ARTICLE

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# Circular RNA MTO1 intercorrelates with microRNA-630, both associate with Enneking stage and/or pathological fracture as well as prognosis in osteosarcoma patients

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## Abstract

**Objective:** Circular RNA-mitochondrial tRNA translation optimization 1 (circ-MTO1) not only involves in bioprocess of various cancers, but also regulates osteosarcoma progression by regulating microRNA-630 (miR-630). However, the clinical role of circ-MTO1 and miR-630 in osteosarcoma is still obscure. This study aimed to assess the correlation of circ-MTO1 and miR-630 with disease features and prognosis and to explore their association with each other in osteosarcoma patients.

**Methods:** Forty-four osteosarcoma patients who received neoadjuvant chemotherapy to surgical resection were analyzed in this retrospective study. Then, circ-MTO1 and miR-630 expressions were evaluated in tumor and adjacent non-tumor specimens by reverse transcription quantitative polymerase chain reaction.

**Results:** Circ-MTO1 was lower in tumor than in non-tumor tissues ($p<0.001$); meanwhile, its elevated tumor expression was correlated with less advanced Enneking stage ($p=0.049$), good neoadjuvant chemotherapy response ($p=0.029$), and longer disease-free survival (DFS) ($p=0.047$). However, no association was found between circ-MTO1 and overall survival (OS) ($p=0.122$). Additionally, miR-630 in tumor was higher than in non-tumor tissues ($p<0.001$), while its raised tumor expression was associated with pathological fracture occurrence ($p=0.003$), advanced Enneking stage ($p=0.036$), poor neoadjuvant chemotherapy response ($p=0.035$), and shorter DFS ($p=0.011$). However, no association was found between miR-630 and OS ($p=0.066$). In addition, tumor circ-MTO1 was negatively associated with miR-630 ($r=-0.323$, $p=0.032$).

**Conclusion:** Circ-MTO1 and miR-630 expressions are inter-correlated and dysregulated in osteosarcoma patients. Besides, they associate with Enneking stage and/or pathological fracture, as well as neoadjuvant treatment response and accumulating DFS in these patients.

**KEYWORDS**
circular RNA MTO1, Enneking stage, microRNA-630, osteosarcoma, Prognosis

Zhihua Shi and Ye Wen contributed equally to this work.

## 1 | INTRODUCTION

Osteosarcoma is a bone tumor that arises from mesenchymal cells forming osteoid and immature bones, which most commonly affects children and young adults.[1,2] It often occurs during the adolescent growth spurt with an incidence of eight to eleven million per year at the age of 15–19 years in 2016.[1,3,4] Although its overall incidence is low, osteosarcoma has relatively high disease burden, including pain, swelling, and pathologic fracture. Besides, most osteosarcoma patients are high-grade intramedullary or surface subtypes at diagnosis who always miss the appropriate opportunity for surgery.[5,6] For these patients, neoadjuvant chemotherapy greatly improves the limb salvage rate, disease control rate and provides the possibility of surgery.[7] However, there are still some osteosarcoma patients who appear with poor response to neoadjuvant chemotherapy.[8] To assist in improving the management of osteosarcoma patients, new biomarkers might be needed.

Circular RNA (circRNA) is a novel class of non-coding RNAs presented with closed loops without poly A tails, which plays a vital role in carcinogenesis.[9,10] Among them, circRNA-mitochondrial tRNA translation optimization 1 (circ-MTO1) is previously reported as an anti-tumor gene with a length of 47499 nucleotides located at chromosome 6, which involves in the regulation of many biological activities of tumor cells.[11,12] For instance, through sponge activity of micro ribonucleic acid-9 and consequently upregulating p21, circ-MTO1 suppresses hepatocellular carcinoma (HCC) cell proliferation and invasion, while enhances apoptosis[13]; additionally, via activating Wnt/β-catenin signaling pathway, circ-MTO1 inhibits the proliferation and invasion of colorectal cancer (CRC) cells, thus participating the progression of CRC.[14] In terms of osteosarcoma, a study reveals that circ-MTO1 regulates the proliferation and metastasis of osteosarcoma cells via microRNA-630 (located at chromosome 15 with the length of 96 nucleotides)/kruppel-like factor 6 (miR-630/KLF6) axis.[15] Based on the above information, we hypothesized that circ-MTO1 and miR-630 might be potential biomarkers for osteosarcoma.

The current study was objective to investigate the relation between circ-MTO1 and miR-630, and their correlation with clinical features, neoadjuvant chemotherapy response, disease-free survival (DFS), and overall survival (OS) in osteosarcoma patients.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients

This retrospective study reviewed 44 osteosarcoma patients who received surgical resection in the hospital from January 2017 to February 2021. The screening criteria included (i) diagnosis of osteosarcoma by pathological findings; (ii) Enneking stage IIA or IIB, and received neoadjuvant chemotherapy; (iii) had complete clinical data and follow-up data; (iv) had available tumor and adjacent non-tumor specimens to perform reverse transcription quantitative polymerase chain reaction (RT-qPCR) assay; and (v) no history of or complication with other primary carcinomas or malignancies before diagnosis. This study was permitted by Institutional Review Board.

### 2.2 | Collection of data and samples

According to the medical documents, the clinical features were obtained, including age, gender, pathological fracture, tumor location, classification of sarcoma, Enneking stage, surgery type, and treatment information. For samples, 44 pairs of tumor and adjacent non-tumor specimens which were frozen in liquid nitrogen were collected to evaluate the circ-MTO1 and miR-630 expression in the study.

### 2.3 | RT-qPCR assay

After collection of frozen specimens, the expressions of circ-MTO1 and miR-630 were determined by RT-qPCR. The specimens were treated by RNeasy Protect Mini Kit (Qiagen, Duesseldorf, Nordrhein-Westfalen, Germany) to extract total RNA. The linear RNA was removed from the total RNA using RNase R (Epicentre, Madison, Wisconsin,USA) in the detection of circRNA expression. Reverse transcription was performed using PrimeScript™ RT reagent Kit (Perfect Real Time) (Takara, Dalian, Liaoning, China). After that, qPCR was carried out with KOD SYBR® qPCR Mix (Toyobo, Osaka, Kansai, Japan). GAPDH and U6 were served as reference genes of circ-MTO1 and miR-630, respectively. The quantitative analyses of circ-MTO1 expression and miR-630 expression were conducted with the use of $2^{-\Delta\Delta Ct}$ method. The primers used in qPCR were designed in accordance with the previous study.[15]

### 2.4 | Neoadjuvant treatment response and follow-up

The treatment records of patients indicated that all patients received preoperative neoadjuvant chemotherapy with high-dose of methotrexate and cisplatin combined with doxorubicin for two cycles, and then, the patients underwent surgical resection. The tumor necrosis rate was obtained from the postoperative pathological examination documents. For patients with good response (tumor necrosis rate ≥90%), they continued the original neoadjuvant regimen for adjuvant therapy; as for patients with poor response (tumor necrosis rate <90%), they received the enhanced adjuvant chemotherapy or other treatment regimen. In addition, survival information of patients was collected from the follow-up records. To the current study, the final follow-up date was April 2021. DFS and OS were calculated on the basis of the recorded data.

### 2.5 | Statistical analysis

SPSS V.19.0 software (IBM Corp., Armonk, New York, USA) and GraphPad Prism 7.02 (GraphPad Software Inc., San Diego, California, USA) were used for statistical analyses and graph plotting. Wilcoxon signed-rank test was applied to compare the circ-MTO1 and miR-630 expression between tumor and paired adjacent non-tumor specimens. Correlation of circ-MTO1 expression with miR-630 expression was assessed using Spearman's rank correlation test. Correlations of circ-MTO1 and miR-630 expression with clinical features and response rate were evaluated by Wilcoxon rank sum test or Kruskal-Wallis H rank sum test. Correlations between the RNA expression and survival data were evaluated by Kaplan-Meier curves and log-rank test. Statistical significance was concluded if a $P$ value <0.05 was presented in the corresponding analysis.

## 3 | RESULTS

### 3.1 | Clinical features

A total of 44 osteosarcoma patients who received neoadjuvant chemotherapy to surgical resection were analyzed in this study, with their characteristics summarized in Table 1. In brief, the mean age of osteosarcoma patients was 21.0±14.3 years. There were 20 (45.5%) female patients and 24 (54.5%) male patients. The number of patients with or without pathological fracture was 8 (18.2%) and 36 (81.8%), respectively. Regarding WHO classification of sarcoma, 25 (56.8%) patients were classified as conventional osteoblastic sarcoma, 7 (15.9%) patients were classified as conventional chondroblastic sarcoma, 6 (13.6%) patients were classified as other conventional sarcoma, and 6 (13.6%) patients were classified as telangiectatic sarcoma. Additionally, the number of patients at Enneking stage IIA and stage IIB were 11 (25.0%) and 33 (75.0%), respectively. Furthermore, as to surgery type, 29 (65.9%) patients received limb salvage surgery and 15 (34.1%) patients received amputation surgery.

**TABLE 1** Clinical features

| Items | Osteosarcoma patients (N = 44) |
|---|---|
| Age (years), mean±SD | 21.0±14.3 |
| Gender, No. (%) | |
| Female | 20 (45.5) |
| Male | 24 (54.5) |
| Pathological fracture, No. (%) | |
| No | 36 (81.8) |
| Yes | 8 (18.2) |
| Location, No. (%) | |
| Femur | 23 (52.3) |
| Tibia | 14 (31.8) |
| Others | 7 (15.9) |
| WHO classification of sarcoma, No. (%) | |
| Conventional: osteoblastic | 25 (56.8) |
| Conventional: chondroblastic | 7 (15.9) |
| Conventional: other | 6 (13.6) |
| Telangiectatic | 6 (13.6) |
| Enneking stage, No. (%) | |
| Stage IIA | 11 (25.0) |
| Stage IIB | 33 (75.0) |
| Surgery type, No. (%) | |
| Limb salvage | 29 (65.9) |
| Amputation | 15 (34.1) |

Abbreviations: SD, standard deviation; WHO, World Health Organization.

### 3.2 | Circ-MTO1 expression and miR-630 expression in tumor and adjacent non-tumor tissues

Circ-MTO1 expression was lower in tumor tissue (N=44) than in non-tumor tissue (N=44) ($p$<0.001) (Figure 1A). Meanwhile, miR-630 expression in tumor tissue (N=44) was increased than that in non-tumor tissue (N=44) ($p$<0.001) (Figure 1B).

### 3.3 | Correlation of circ-MTO1 expression with miR-630 expression in tumor tissue

The association of circ-MTO1 expression with miR-630 expression in osteosarcoma tumor tissue was investigated subsequently, and data showed that circ-MTO1 expression was negatively associated with miR-630 expression in tumor tissue ($r$=−0.323, $p$=0.032) (Figure 2).

### 3.4 | Association of circ-MTO1 expression and miR-630 expression in tumor tissue with clinical features

Elevated circ-MTO1 expression was correlated with lower Enneking stage ($p$=0.049). In addition, elevated miR-630 expression was associated with occurrence of pathological fracture ($p$=0.003); meanwhile, it was correlated with higher Enneking stage ($p$=0.036). However, no association was found in circ-MTO1 expression or miR-630 expression with other clinical features (all $p$>0.05) (Table 2).

### 3.5 | Correlation of circ-MTO1 expression and miR-630 expression in tumor tissue with prognosis

Elevated circ-MTO1 expression was associated with good neoadjuvant treatment response ($p$=0.029) (Table 3); meanwhile, circ-MTO1 high expression was correlated with longer accumulating DFS ($p$=0.047) (Figure 3A). However, no association was found in

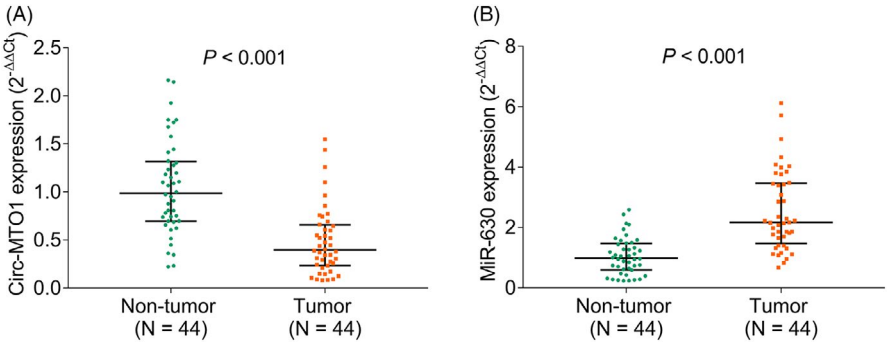


**FIGURE 1** Circ-MTO1 expression and miR-630 expression in osteosarcoma patients. Comparison of circ-MTO1 expression (A) and miR-630 expression (B) between the tumor tissue and non-tumor tissue. circ-MTO1, circular RNA-mitochondrial tRNA translation optimization 1; miR-630, microRNA-630

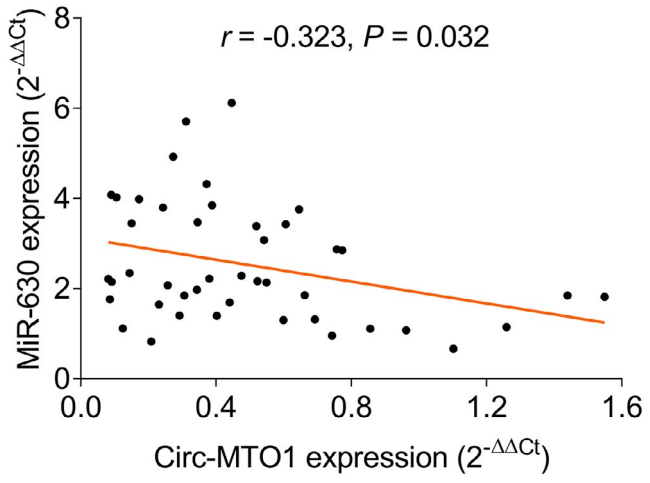


**FIGURE 2** Correlation of circ-MTO1 expression with miR-630 expression in osteosarcoma tumor tissue. circ-MTO1, circular RNA-mitochondrial tRNA translation optimization 1; miR-630, microRNA-630

circ-MTO1 expression with accumulating OS ($p$>0.05) (Figure 3B). In addition, elevated miR-630 expression was correlated with poor neoadjuvant treatment response ($p$=0.035) (Table 3); meanwhile, miR-630 high expression was associated with shorter DFS ($p$=0.011) (Figure 3C). However, no association was found in miR-630 expression with accumulating OS ($p$>0.05) (Figure 3D). According to univariate Cox's regression analysis, MiR-630 high (vs. low) was correlated with poor DFS ($p$=0.016, Hazard Ratio (HR)= 3.432). Meanwhile, according to multivariate Cox's regression analysis, age ≥20 years (vs. <20 years) was independently associated with shorter DFS ($p$=0.037, HR=3.880) and OS ($p$=0.007, HR=13.799). However, no correlation was found in other factors with prognosis according to Cox's proportional hazards regression analysis (all $p$>0.05) (Tables S1-2).

## 4 | DISCUSSION

Some studies reports that circ-MTO1 expression decreases in tumor tissue than in non-tumor tissue, such as gastric cancer (GC), glioblastoma, and osteosarcoma.[15-17] For instance, circ-MTO1 declines in GC tissue than in paired adjacent tissue.[16] Another study reveals that circ-MTO1 expression is lower in glioblastoma tissue than in normal tissue.[17] Besides, circ-MTO1 expression is decreased in osteosarcoma tumor tissue compared with adjacent tissue[15]; in line with previous studies, our study found that circ-MTO1 expression was decreased in tumor tissue than in non-tumor tissue. The explanation could be that: circ-MTO1 could reflect the lower proliferation rate of cells, meanwhile, the proliferation rate in osteosarcoma cells was higher than that in the adjacent tissue cells. Therefore, circ-MTO1 expression was lower in tumor tissue than in non-tumor tissue. Moreover, regarding the correlation of circ-MTO1 expression with disease feature, elevated circ-MTO1 expression associates with aggravating clinical features in solid tumors.[11,18] In our study, we found that elevated circ-MTO1 expression in tumor tissue was correlated with milder disease feature. The reason might be that: circ-MTO1 attenuated the growth of osteosarcoma cancer cells, thus inhibiting the progression of osteosarcoma and resulting in favorable disease feature.[15,16] In terms of the correlation of circ-MTO1 expression with prognosis, recent results suggest that increased circ-MTO1 expression correlates with better survival profile in prostate cancer and osteosarcoma.[11,15] For instance, a study shows that circ-MTO1 expression independently correlates with prolonged DFS in prostate cancer patients.[11] Another study suggests that circ-MTO1 expression is associated with longer OS in osteosarcoma patients.[15] Our study also discovered that: (1) elevated circ-MTO1 expression was correlated with good neoadjuvant treatment response, a possible explanation could be that: elevated circ-MTO1 expression might improve the sensitivity of osteosarcoma cells to chemotherapeutic drugs, thus it was related to good treatment response, which needed further validation by experiments; (2) circ-MTO1 high was associated with better accumulating DFS, one explanation could be that: circ-MTO1 high was associated with favorable disease feature as mentioned above, which might indirectly cause longer DFS.

Regarding the clinical role of miR-630 wiin cancers, some interesting studies have performed to investigate its association with clinical characteristics in epithelial ovarian cancer and colorectal cancer.[19,20] For example, it is revealed that miR-630 expression is upregulated in epithelial ovarian cancer tissue compared with that of the normal ovarian tissue, while it correlates with more advanced tumor-node-metastasis (TNM) stage and poorer prognosis in ovarian cancer patients.[20] Besides, miR-630 expression increases in colorectal cancer specimens than adjacent specimens; elevated miR-630

**TABLE 2** Correlation of circ-MTO1 and miR-630 expressions with clinical features

| Items | Circ-MTO1 expression Median (IQR) | *P* value | MiR–630 expression Median (IQR) | *P* value |
|---|---|---|---|---|
| Age | | 0.283 | | 0.392 |
| < 20 years | 0.440 (0.273–0.692) | | 1.975 (1.323–3.453) | |
| ≥ 20 years | 0.311 (0.158–0.597) | | 2.224 (1.709–3.782) | |
| Gender | | 0.654 | | 0.239 |
| Female | 0.376 (0.224–0.632) | | 2.064 (1.343–3.312) | |
| Male | 0.443 (0.234–0.719) | | 2.317 (1.728–3.840) | |
| Pathological fracture | | 0.224 | | 0.003 |
| No | 0.421 (0.247–0.730) | | 1.917 (1.343–3.030) | |
| Yes | 0.292 (0.156–0.510) | | 3.873 (2.578–4.703) | |
| Location | | 0.213 | | 0.619 |
| Femur | 0.388 (0.150–0.606) | | 2.220 (1.406–3.430) | |
| Tibia | 0.376 (0.254–0.529) | | 2.153 (1.858–3.873) | |
| Others | 0.692 (0.311–0.962) | | 1.323 (1.079–3.985) | |
| WHO classification of sarcoma | | 0.980 | | 0.185 |
| Chondroblastic | 0.446 (0.090–0.599) | | 3.389 (1.821–4.928) | |
| Osteoblastic | 0.380 (0.220–0.750) | | 1.859 (1.236–2.865) | |
| Other | 0.458 (0.273–0.616) | | 2.858 (1.814–3.817) | |
| Telangiectatic | 0.427 (0.205–0.687) | | 2.618 (1.769–4.280) | |
| Enneking stage | | 0.049 | | 0.036 |
| Stage IIA | 0.542 (0.402–0.962) | | 1.697 (1.148–2.224) | |
| Stage IIB | 0.343 (0.190–0.626) | | 2.286 (1.795–3.828) | |
| Surgery type | | 0.577 | | 0.785 |
| Limb salvage | 0.388 (0.190–0.622) | | 2.220 (1.404–3.620) | |
| Amputation | 0.519 (0.243–0.743) | | 2.136 (1.650–3.430) | |

Abbreviations: Circ-MTO1, circular RNA-mitochondrial tRNA translation optimization 1; IQR, interquartile range; miR-630, microRNA-630; WHO, World Health Organization.

**TABLE 3** Correlation of circ-MTO1 and miR-630 expressions and neoadjuvant treatment response

| Items Median (IQR) | Good response patients (n = 17) | Poor response patients (n = 27) | *P* value |
|---|---|---|---|
| Circ-MTO1 expression | 0.440 (0.325–1.032) | 0.372 (0.144–0.599) | 0.029 |
| MiR–630 expression | 1.853 (1.362–2.197) | 2.856 (1.768–3.852) | 0.035 |

Abbreviations: Circ-MTO1, circular RNA-mitochondrial tRNA translation optimization 1; IQR, interquartile range; miR-630, microRNA-630.

expression in tumor tissue correlates with higher TNM stage and shorter OS.[19] In our study, we found that: (1) miR-630 expression in tumor tissue was higher than that in non-tumor tissue, a possible explanation might that: miR-630 reflected the proliferation rate of cells, and meanwhile, the proliferation rate in osteosarcoma cells was higher than that in the adjacent tissue cells. Therefore, miR-630 expression was higher in tumor tissue than in non-tumor tissue; (2) elevated miR-630 was correlated with the occurrence of pathological fracture and deteriorated disease severity feature, the reason might be that: elevated miR-630 promoted malignant osteosarcoma cell proliferation,[21] which might contribute to the osteosarcoma progression, subsequently causing bone lesion and deteriorated disease feature[6]; (3) elevated miR-630 expression was associated with poor neoadjuvant treatment response, the explanations could be that: miR-630 expression might increase cisplatin resistance in osteosarcoma,[22] thus causing unfavorable treatment response; (4) miR-630 high was associated with shorter DFS, a possible reason could be that: its association with deteriorated disease feature and unfavorable treatment response (as above mentioned) might indirectly resulted in poor DFS.

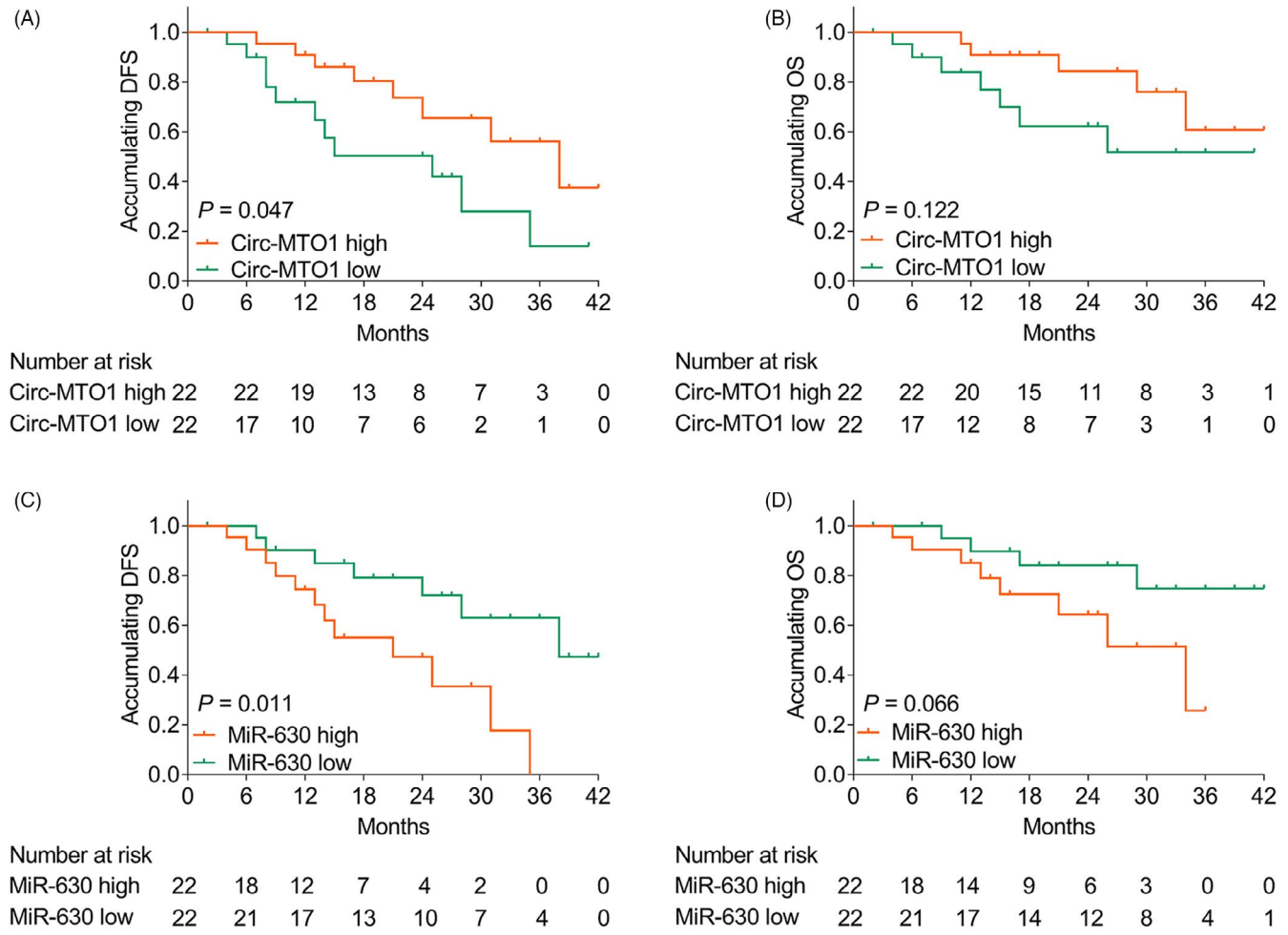


**FIGURE 3** Association of circ-MTO1 expression and miR-630 expression with accumulating DFS and OS in osteosarcoma patients. Association of circ-MTO1 expression (A) and miR-630 expression (C) with accumulating DFS; association of circ-MTO1 expression (B) and miR-630 expression (D) with accumulating OS. circ-MTO1, circular RNA-mitochondrial tRNA translation optimization 1; DFS, disease-free survival; OS, overall survival; miR-630, microRNA-630

In terms of the association of circ-MTO1 expression with miR-630 expression in osteosarcoma patients, as far as we knew, there was no study conducted before to explore the association between them. Our study observed that circ-MTO1 was negatively correlated with miR-630 expression in osteosarcoma patients, a possible reason might be that circ-MTO1 expression served as a sponge for miR-630 expression,[15] which needed further validation.

Although a lot of findings were identified, there were still some limitations in this study. Firstly, though the incidence of osteosarcoma was low, we had tried to enroll more patients in this study. However, the sample size was still relatively small, which might cause low statistical power. Secondly, this study did not investigate the molecular mechanism of circ-MTO1 or miR-630 involved in the osteosarcoma progression, and thus, experiments might be further conducted in osteosarcoma patients. Thirdly, this study existed some confounding factors, for example, patients might have received adjuvant chemotherapy after surgery, which might affect the prognosis of patients to some extent. Fourthly, subsequent experiments were needed to investigate the function of miR-630 in regulating osteosarcoma cell.

Conclusively, circ-MTO1 and miR-630 expressions are intercorrelated and dysregulated in osteosarcoma patients. Besides, they associate with Enneking stage and/or pathological fracture, as well as neoadjuvant treatment response and accumulating DFS in these patients. They might potentially serve as biomarkers for osteosarcoma prognosis, which improve the management of osteosarcoma patients.

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## CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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## SUPPORTING INFORMATION

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