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The Roles of Circular RNAs in Osteosarcoma

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Osteosarcoma is a malignant tumor that occurs most commonly in the metaphysis of the long bones in the limbs in children and adolescents. Even with surgery and neoadjuvant chemotherapy, the therapeutic effect has reached a peak with 60–70% survival rates. Therefore, new biological targets or molecular mechanisms that enhance the efficacy of osteosarcoma treatments are needed. Circular RNAs (circRNAs) are useful biomarkers that have recently been recognized clinically and in medical research and have been of interest due to the use of next-generation sequencing and bioinformatics analysis. CircRNAs are involved in many diseases, including cancer. Therefore, this review aims to summarize the roles of circRNA in the diagnosis, progression, and prognosis of osteosarcoma.

MeSH Keywords: Osteosarcoma • Review • RNA, Ribosomal

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Background

Osteosarcoma is a malignant tumor that is most common in the metaphyses of limbs in both children and teenagers [1], and the distal femur, humerus, and proximal tibia are hot spots [2]. Also, in adolescents, osteosarcoma has a second peak in the population over 50 years old. Osteosarcoma consists of malignant osteoblasts that produce immature bone or osteoid tissue. The treatment of osteosarcoma consists of surgery in early stages and adjuvant chemotherapy (began in the 1970s) to neoadjuvant chemotherapy, which is the present standard therapy [3]. Also, the cure rates of patients with non-metastatic osteosarcoma were less than 20% [4], which increased to almost 50% [5] and are currently 70%. However, patients with metastasis and chemotherapy drug resistance suffer from dismal survival rates [6], and lung metastasis is the most frequent complication. Therefore, to enhance the efficacy of osteosarcoma treatment, new biological targets or new molecular mechanisms are needed. CircRNA has recently been recognized as a useful biomarker by clinicians and researchers.

CircRNA acts as a microRNA (miRNA) sponge and is a novel endogenous RNA that competes for microRNAs and long noncoding RNAs [7]. CircRNA was first identified in the 1970s and is characterized by covalently closed loop structures and the lack of 3' and 5' polyadenylated tails [8,9]. Therefore, circRNA is more conservative and stable than the corresponding linear RNA [10]. CircRNAs come from different sources and produce different circRNAs [11]. For example, circRNAs can originate from coding precursor mRNA (pre-mRNA), noncoding mRNA (ncRNA), mature mRNA, intron lariats, and intergenic genes. The types of circRNAs include exonic circular RNA (ecircRNA), exon-intron circRNA (ElciRNA), intergenic circRNA, and antisense circRNA. Initially, circRNA did not receive attention from researchers and was considered to be the subsidiary products of splicing errors. With improved next-generation sequencing and bioinformatics analysis, increased research on circRNA has been conducted. Many circRNAs are involved in the progression of multiple diseases, especially tumorigenesis [12], and their characteristics of conservation, stability, specificity, richness, and easy detection were recognized [13]. Hence, circRNA is considered a new and useful tumor biomarker [14,15]. In this review, we summarize the roles of circRNA in the progression, prognosis, and diagnosis of osteosarcoma.

CircRNAs in the Progression of Osteosarcoma

To investigate the functions of circRNAs in the progression of osteosarcoma, researchers usually conduct a circRNA microarray analysis of osteosarcoma tissues and cell lines or screen microarray datasets to identify a novel circRNA that may be involved in the proliferation of osteosarcoma cells. Considering that circRNAs function as a miRNA sponge, it is essential to filter the target miRNAs of circRNAs. Bioinformatics tools such as miRBase predict the potential target miRNAs and are verified by luciferase reporter assays. The relationship between circRNA and selected target miRNA has been further confirmed by loss-of-function and gain-of-function analysis, following which the oncogenic function of the circRNA has been confirmed by rescue assays to support the experimental findings.

The oncogenic function of overexpressed circRNA involves promotion of cell proliferation, colony formation, and invasion and reduction in the apoptotic cell rate. For example, CircRNA GLI2 positively affects cell proliferation, invasion, and migration and negatively affects apoptosis. The oncogenic function of downregulated circRNAs such as circ_0002052 contrasts that of overexpressed circRNAs. Some investigators have generated xenografts of osteosarcoma cells in nude mice and found that overexpression of certain circRNAs, such as circ_0001721 and circ_0000502, promoted tumor volume and weight increase. Also, circ_0009910, circRNA NASP, and circ_0001564 were confirmed to affect G0/G1 arrest negatively. Furthermore, circRNA can act through some tumor-related pathways and plays a carcinogenic role. For example, a rescue assay showed that the oncogenic function of circ_0009910 partly depends on the JAK1/STAT3 pathway, which is involved in apoptosis and proliferation [16]. CircRNAs that affect the progression of osteosarcoma are described in Table 1.

CircRNAs in the Prognosis of Osteosarcoma

To determine the relationship between circRNA expression and prognosis in osteosarcoma, correlation analysis has shown that the expression of circRNA in osteosarcoma was related to certain prognostic factors, such as tumor size, Enneking stage, and the occurrence of distant metastasis. For example, Fisher's exact test showed that the level of circ_0007534 was related to tumor size and degree of differentiation or tumor grade, but not to the World Health Organization (WHO) stage and in advanced osteosarcoma associated with pulmonary metastasis. For circ_0000502, all tumor sizes, WHO grades and stage, and pulmonary metastasis rates in the overexpression group were higher than those in the low-expression group. CircRNA CDR1 and circ_001569 were upregulated in OS, and both were associated with tumor size, Enneking stage (or TNM stage), and pulmonary metastasis but not patient age, or gender.

Neoadjuvant chemotherapy is currently the standard therapy for osteosarcoma, and chemoresistance can influence the prognosis of osteosarcoma. Zhang et al. found that suppression of circ_001569 activity in osteosarcoma cell lines led to decreased IC50 values for doxorubicin, methotrexate, or cisplatin, and Wnt/β-catenin pathway agonist rescued the decreased IC50 values. Additionally, the Wnt/β-catenin pathway is reportedly involved

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Table 1. Characterization of circRNAs that affect the progression of osteosarcoma.

CircRNA	Deregulation	Genes/proteins affected	Roles
Circ_0008717 [17]	Overexpression	miR-203/Bmi-1	Proliferation (+), invasion (+), migration (+), apoptosis (–)
Circ_0009910 [18]	Overexpression	miR-449a/IL6R/JAK1-STAT3	Proliferation (+), apoptosis (–), G0/G1 arrest (–)
CircRNA_GLI2 [19]	Overexpression	miR-125b-5p	Proliferation (+), invasive (+), migration (+), apoptosis (–)
CircRNA_NASP [20]	Overexpression	miR-1253/FOXF1	Proliferation (+), G0/G1 arrest (–)
Circ_0001721 [21]	Overexpression	miR-569 and miR-599	Proliferation (+), tumor volume (+), tumor weight (+), apoptosis (–)
Circ_0007534 [22]	Overexpression	AKT/GSK-3β pathway, Bcl-2/caspase-3 pathway	Proliferation (+), invasion (+), tumor weight (+), apoptosis (–)
Circ_0002052 [23]	Low-Expression	miR-1205/APC2/Wnt/β-catenin	Apoptosis (+), tumor growth (–), proliferation (–)
circ_0000502 [24]	Overexpression	miR-1238	Migration (+), proliferation (+), invasion (+), tumor volume (+), tumor weight (+), apoptosis (–)
Circ_001569 [25]	Overexpression	/	Proliferation (+), clone formation (+)
circRNA_CDR1as [26]	Overexpression	miR-7	Cell vitality (+), migration (+), tumor growth (+), G1/S arrest (–)
circ-0016347 [27]	Overexpression	miR-214/caspase-1	Oncogenic ability (+)
Circ_0001564 [28]	Overexpression	miR-29c-3p	Proliferation (+), G0/G1 arrest (–), apoptosis (–)
Circ-NT5C2 [29]	Overexpression	miR-448	Proliferation (+), invasion (+), tumor growth (+)
CircFAT1 [30]	Overexpression	miR-375/YAP1	Proliferation (+), migration (+), apoptosis (–)
CircRNA UBAP2 [31]	Overexpression	miR-143/Bcl-2	Oncogenic ability (+)

in osteosarcoma chemoresistance [32]. Therefore, circ_001569 may promote cisplatin resistance through the Wnt/β-catenin pathway. Circ_0081001 was selected from chemoresistant and chemosensitive osteosarcoma cell lines, and further experiments demonstrated that circ_0081001 levels were higher in advanced Enneking stage patients, chemoresistant patients, and lung metastasis patients. Thus, circ_0081001 may be a potential biomarker for prognosis of osteosarcoma.

Meanwhile, the relationship between the expression of circRNAs and survival rates, including 5-year survival rates, overall survival time, and disease-free survival time, of osteosarcoma patients was detected by Kaplan-Meier analysis. Circ_0001721 and circ_0000502 were negatively correlated with 5-year survival rates; circRNA UBAP2 and circRNA PVT1 were negatively correlated with overall survival time, whereas circRNA HIPK3 was positively correlated with overall survival time. Moreover, circRNA NT5C2 was negatively correlated with overall survival and disease-free survival. The circRNAs that affect the prognosis of osteosarcoma are described in Table 2.

CircRNAs in the Diagnosis of Osteosarcoma

Circ_0008717 was shown to be upregulated in patients with osteosarcoma. The value of the area under the curve (AUC) was 0.782, and the diagnostic specificity and sensitivity were 0.73 and 0.80, respectively. CircRNA-CDR1as was upregulated in osteosarcoma tissues and cell lines, and receiver operating characteristic (ROC) curve analysis indicated that circRNA-CDR1as was a novel diagnostic marker of osteosarcoma as the value of the AUC reached 0.857. CircRNA-NT5C2 was overexpressed in osteosarcoma tissues, and the AUC value was 0.753. Therefore, circRNA-NT5C2 may be an excellent diagnostic label to distinguish osteosarcoma patients from healthy individuals. CircRNA-HIPK3was downregulated in osteosarcoma

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Table 2. Characterization of circRNAs that affect the prognosis of osteosarcoma.

CircRNA	Deregulation	Genes/proteins affected	Roles
Circ_0008717 [17]	Overexpression	miR-203/Bmi-1	Clinical stage (+), distant metastasis (+), overall survival (–), disease-free survival (–)
Circ_0001721 [21]	Overexpression	miR-569, miR-599	WHO grade (+), tumor size (+), 5-year overall survival (–)
Circ_0007534 [22]	Overexpression	AKT/GSK-3β pathway, Bcl-2/caspase-3 pathway	Tumor size (+), differentiation grade (+), overall survival rates (–)
Circ_0002052 [23]	Overexpression	miR-1205/APC2/Wnt/β-catenin	Clinical stage (+), distant metastasis (+), overall survival (–), disease-free survival (–)
Circ_0000502 [24]	Overexpression	miR-1238	WHO grade (+), tumor size (+), pulmonary metastasis (+), 5-year prognosis (–)
Circ_001569 [25]	Overexpression	/	Distant metastasis (+), TNM stage (+), chemotherapeuti-c resistance (+)
circRNA_ CDR1as [26]	Overexpression	miR-7	Tumor size (+), enneking stage (+), pulmonary metastasis (+)
CircRNA_UBAP2 [31]	Overexpression	miR-143/Bcl-2	Tumor stages (+), overall survival (–)
circ_HIPK3 [33]	Low-expression	/	Overall survival (–), enneking stage (+), lung metastasis (+)
circ-NT5C2 [34]	Overexpression	/	Clinical stage (+), distant metastasis (+), overall survival (–), disease-free survival (–)
CircPVT1 [35]	Overexpression	Gene ABCB1	Lung metastasis (+), chemoresistant (+), enneking stage (+), overall survival (–)
Circ_0081001 [36]	Overexpression	/	Enneking stage (+), lung metastasis (+), chemoresistant (+), overall survival (–)

Table 3. Characterization of circRNAs that could be diagnostic biomarkers of osteosarcoma.

CircRNA	Deregulation	Genes/proteins affected	Diagnostic value
Circ_0008717 [17]	Overexpression	miR-203/Bmi-1	AUC value: 0.782, sensitivity value: 0.8, specificity value: 0.73
circRNA_ CDR1as [26]	Overexpression	miR-7	AUC value: 0.857
Circ-NT5C2 [29]	Overexpression	miR-448	AUC value: 0.753
circ_HIPK3 [33]	Overexpression	/	Cutoff value: 29.3, sensitivity value: 0.56, specificity value: 0.84
CircPVT1 [35]	Overexpression	Gene ABCB1	AUC value: 0.871
Circ_0081001 [36]	Overexpression	/	AUC value: 0.898

tissues and plasma. To explore the diagnostic value of circRNA-HIPK3, a ROC curve was drawn, and the cutoff value was 29.3. The sensitivity value was 56%, and the specificity was 84%. CircRNA-PVT1 was not only upregulated in osteosarcoma tissues but was also overexpressed in sera, making circRNA-PVT1 easy to detect as a diagnostic marker.

Also, the area under the curve (AUC) value of circRNA-PVT1 (0.871) was equivalent to lactate dehydrogenase (LDH) (0.852), which was greater than alkaline phosphatase (ALP) (0.673), both of which are clinical diagnostic markers in osteosarcoma. Zhu et al. also investigated the diagnostic value of circ_0081001, and the AUC value of circ_0081001 was 0.898, which was similar to circPVT1 (0.871). Table 3 lists the circRNAs that could be potential diagnostic biomarkers of osteosarcoma.

CircRNA is more abundant and stable than its liner RNA counterparts. With the improvement of next-generation sequencing

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and bioinformatics analysis, the function of circRNA is increasingly well known, especially in cancers. Recently, circRNA has been identified as a potential novel biomarker and an underlying therapeutic target in osteosarcoma.

Conclusions

In this review, we summarized the function of circRNAs in the progression, prognosis, and diagnosis of osteosarcoma and their predicted downstream miRNAs or potential signaling pathways. However, many circRNAs remain undetected, and their functions in osteosarcoma have not been verified. Also, further studies are needed to investigate the differentially upregulated circRNAs and downregulated circRNAs in osteosarcoma tissues and cell lines. Isolated circRNAs have been reviewed as biomarkers in osteosarcoma, and interactions between or among circRNAs have not been reviewed. Although bioinformatics analysis has identified networks among circRNAs, few studies have confirmed these findings *in vivo* and *in vitro* and further studies are needed.

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