International Journal of General Medicine

Dovepress
open access to scientific and medical research

Open Access Full Text Article

REVIEW

Recent Advances of Circular RNAs as Biomarkers for Osteosarcoma

Hongliang Wu[1–3], Sihang Zheng[4], Qun He[5], Yan Li[3]

[1]Department of Orthopedics, Fuzhou Second Hospital of Xiamen University, School of Medicine, Xiamen University, Xiamen, People's Republic of China; [2]Department of Orthopedics, Fuzhou Second Hospital, Fuzhou, People's Republic of China; [3]Department of Orthopedics, Shengjing Hospital of China Medical University, Shenyang, People's Republic of China; [4]Department of Neurology, Shengjing Hospital of China Medical University, Shenyang, People's Republic of China; [5]Department of Bioinformatics, School of Life Sciences, China Medical University, Shenyang, People's Republic of China

Correspondence: Yan Li; Qun He, Email liy2002@cmu.edu.cn; qhe@cmu.edu.cn

Abstract: Osteosarcoma is the most common primary malignant bone tumor in young adult, which is prone to early metastasis and poor prognosis. The current treatment methods need to be improved. Circular RNA is a covalently blocked circular, non-coding RNA that plays an essential role in the occurrence, development, clinical diagnosis, and treatment of various diseases. Recently, an increasing number of circRNAs have been identified in osteosarcoma. Understanding its role in osteosarcoma is conducive to the early detection, diagnosis, and treatment of osteosarcoma. In this paper, we reviewed the mechanism of action of circular RNA in the occurrence and development of osteosarcoma and its clinical application in recent years.

Keywords: osteosarcoma, circular RNA, biomarker, exosome

Introduction

Osteosarcoma is the most common primary malignant bone tumor in young adult, which is prone to early metastasis and poor prognosis. Because the early diagnosis rate is still low, clinical diagnosis and treatment delay seriously affects patients' diagnosis, treatment, and prognosis. Compared with invasive procedures such as tissue biopsy, liquid biopsy is portable and straightforward and can be used in early screening and prognosis analysis. Therefore, it is of great significance to find biomarkers for the early diagnosis of osteosarcoma. Circular RNA (circRNA) is a covalently blocked noncoding RNA. There are differences in expression profiles between osteosarcoma patients and healthy people. It is closely related to the occurrence, development, metastasis, and drug resistance of osteosarcoma. It suggests that target regulation of circRNA may become a breakthrough in the treatment of osteosarcoma. Liquid biopsy, circRNA-based prognosis analysis, circRNA-based targeted therapy, etc will be a breakthrough in the individualized diagnosis and treatment of osteosarcoma. In this paper, we reviewed the mechanism of circRNA in the development of osteosarcoma and its clinical application in recent years.

Definition of Circular RNA

Circular RNA (circRNA) is a covalently blocked endogenous non-coding RNA. It was first found in plant viroids,[1] yeast mitochondrial RNA,[2] and δ Hepatitis B virus.[3] Later, circRNAs were accidentally found in exons or introns, which were considered by-products of splice body mediated splicing errors. With the further researches, their functions are gradually discovered. A new type of endogenous non-coding RNA molecule is circRNA, which widely and stably exists in eukaryotic cells. It does not have a 5' terminal cap and a 3' terminal poly (a) tail and forms a closed-loop structure with covalent bonds. Genome-wide annotation shows that circRNA is widely expressed in different cell lines and species,[3,4] with different expression levels, and is widely involved in the occurrence and development of various diseases such as cancer.

Received: 2 July 2022
Accepted: 30 November 2022
Published: 15 January 2023

Formation of Circular RNA

Different from the standard splicing of linear RNA connecting upstream (5 ‘) splice donor site with downstream (3’) splice acceptor site, circRNA is produced by reverse splicing of precursor mRNA (connecting downstream splice donor site with upstream splice acceptor site) or exon jumping.[5] CircRNA is cyclized into a continuous closed loop structure, lacking poly(a) tail, and a 5 ‘cap. According to its composition, circRNA can be divided into four categories: exonic circRNA (ecircRNA), exon-intron circRNA (EIciRNA), circular intronic RNA (ciRNA), and tRNA intronic circRNA (tricRNA). At present, three mechanisms of circRNA biogenesis have been widely accepted: RNA binding protein (RBP)-mediated cyclization, intron pairing driven cyclization, and lasso protein driven cyclization. RBP mediated and intron pairing driven cyclization of circRNA occurs through direct reverse splicing. RBP plays an essential role in promoting circRNA biogenesis by regulating adjacent splice sites. In addition, cyclization can be achieved by including reverse complementary sequences. Finally, we promote lasso-driven cyclization by exon jumping events. Internal splicing helps remove the flanking intron sequence and produce ecircRNA; If these flanking sequences are retained, the construct is called EIciRNAs. In addition, ciRNA is generated by lasso-driven cyclization. TricRNA is produced by combining released intron ends produced from spliced pre-tRNA by tRNA splicing endonuclease complex.[6]

Molecular Characteristics of Circular RNA

CircRNAs were first identified in RNA viruses in 1976. They were once considered as “splicing noise” in organisms. With the development of high-throughput sequencing technology and bioinformatics, they have increasingly become a research hotspot. Unlike linear RNA, due to the lack of poly (a) tail, circRNA is stable and resistant to exonuclease (including RNAse R). Its half-life is longer than its corresponding linear RNA, and it can stably exist in various tissues and organs.[7] Its covalent closed loop structure makes it stable in plasma and exosomes. It can be used as a diagnostic marker of tumors and other diseases.[8]

Identification of Circular RNA

In recent years, a new method, RNAse R processing, has been developed to enrich high purity circRNA by polyadenylation and poly (a) + RNA depletion (RPAD). In this method, RNAse R is first used to treat total RNA to deplete linear RNA. The remaining 3 ‘- OH terminal RNA was polyadenylated, consumed by poly (a) + RNA, and passed through oligomeric (DT) beads. RPAD method eliminates the interference of linear RNA and dramatically improves data reliability.[9] The presence of circRNA could not be guaranteed by the circRNA ligation detected by qPCR. If two consecutive exons have very similar sequences or a given RNA is a transcribed product that leads to exon replication or reorganization, these atypical splicing points may originate from linear RNA.[10]

Moreover, template conversion activity from reverse transcriptase can also lead to repetitive sequences in cDNA. Therefore, circRNA validation is a key step in any circRNA research. Strict circRNA verification usually requires 3 ‘exonuclease RNAse R and Northern blotting. Compared with RT qPCR, this is a more accurate method to fully express the RNA types in a given exon.[11] Although RT qPCR is a better quantitative method, it can not identify the true circRNA. It is essential that, since RNase R processing results in a significant change in the concentration and composition of RNA mixtures to be RT-qPCR, adequate internal controls (ie, incorporation of known amounts of foreign RNA) must be used to evaluate RNase R sensitivity of specific circRNA candidates. Another essential step in circRNA validation is to confirm the existence of trans-splicing by Sanger sequencing.

Function of Circular RNA

Circular RNA has many functions in cells[12,13] (Figure 1). (A) CircRNA acts as a miRNA sponge with multiple miRNA binding sites that indirectly control gene expression and play a competitive endogenous RNA role. (B) CircRNA acts as a transcriptional regulator by binding to RNA polymerase II. (C) CircRNA interacts with proteins. In humans and Drosophila, RBP (MBL) binds to circRNA, which plays a role in gene regulation by competing with linear splicing.[14] In mice, cell cycle-related proteins bind to circRNA to enhance p21/CDK2 interaction and block cell cycle progression.[15]

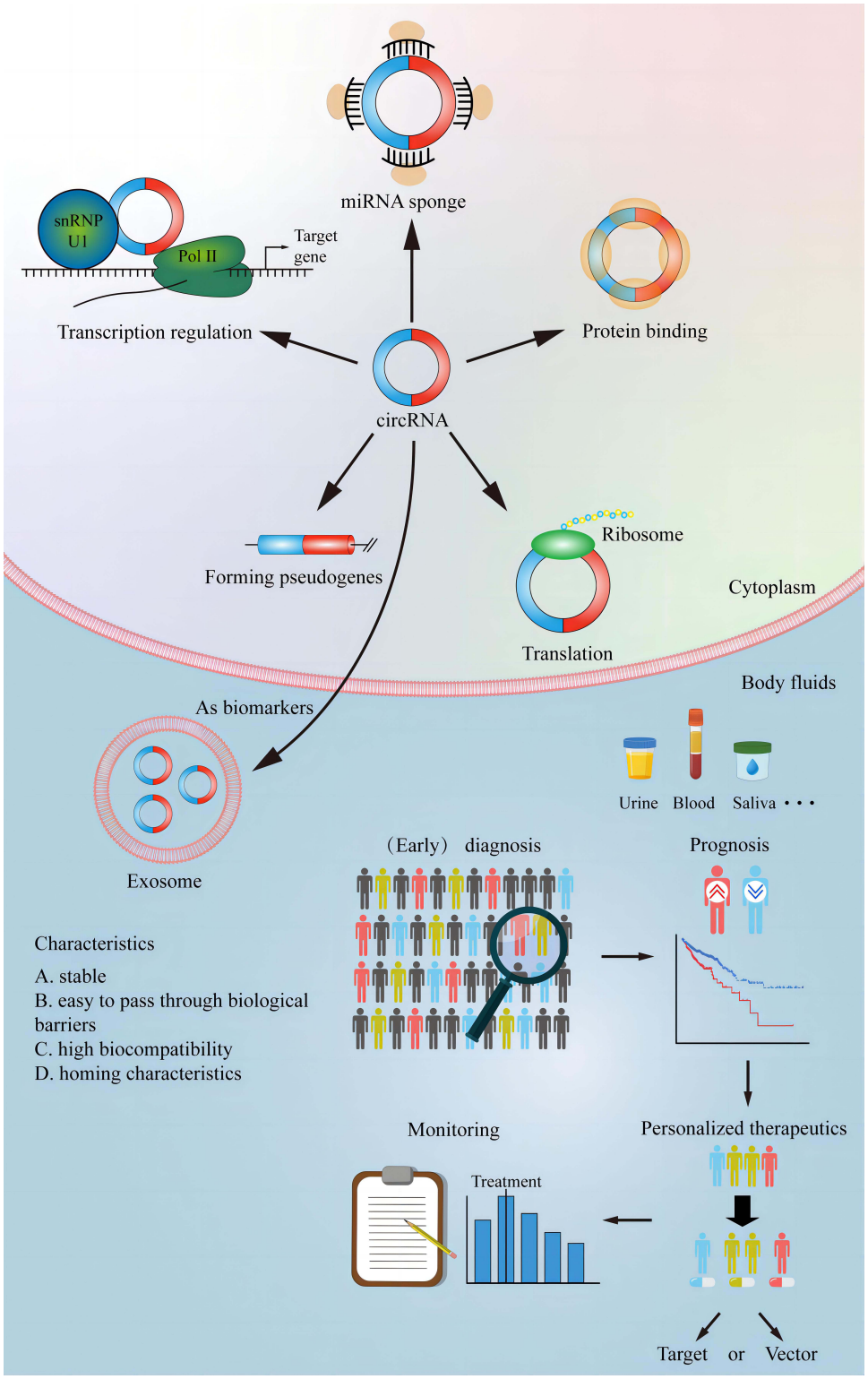

Figure 1 Function of circular RNA; potential application of circular RNA in the diagnosis and treatment of osteosarcoma.

(D) CircRNA containing open reading frame (ORF) and stop codon in the frame is translated into protein in a splicing dependent and cap-dependent manner. (E) Other functions: regulating intercellular signaling pathways, affecting cell differentiation, forming pseudogenes, and other functions.[16–18]

The Role and Regulatory Mechanism of Circular RNA in the Occurrence and Development of Osteosarcoma

Osteosarcoma (OS) is one of the most common malignant bone tumors. It originates from bone mesenchymal cells. The production of a bone-like matrix characterizes it and occurs in adolescents aged 10–20 years old. The most common sites are the distal femur, proximal tibia, and proximal humerus metaphysis. It is highly malignant and prone to local recurrence and metastasis. The prognosis of patients is poor. Somatic mutation and epigenetic mechanism play an important role in abnormal activation of oncogenes (such as cps3, PIK3CA, etc.) and inactivation of tumor suppressor genes (such as TP53, BRCA1/2, etc.).[19] In addition to the changes in these protein-coding genes, the role and regulatory mechanism of circRNA in the occurrence and development of osteosarcoma have become a hot spot in recent research (Table 1).

Carcinogenic Circular RNA

CircTCF25

The first discovery of circTCF25 is the carcinogenic cyclic RNA in bladder cancer.[20] Functionally, the higher expression of circTCF25 promotes the proliferation, migration, and invasion of osteosarcoma cells, accompanied by the corresponding changes of phenotype-related proteins (up-regulation of CyclinD1 and Cdk6 to increase cell proliferation; up-regulation of CyclinD1 and Cdk6 to increase cell proliferation; up-regulation of CyclinD1 and Cdk6 to increase cell proliferation); Up-regulation of MMP2, MMP9, and vimentin, down-regulation of TIMI-1 enhance cell migration and

Table 1 Expression and Association of CircRNAs in Osteosarcoma

CircRNA	Dysregulation	Functions/Mechanisms	Genes/Proteins Affected	Signaling Pathway	(Refs.)
CircTCF25	Up	Promotes the proliferation, migration, and invasion	Upregulation: cyclinD1/CDK6/MMP2/MMP9/vimentin; down-regulation: miR-206/TIMI-1	MEK/ERK, AKT/mTOR	[21]
CircMMP9	Up	Higher viability, colony-forming ability, migration and invasion ability, TMN stage, the lower overall survival rate	Upregulation: CHI3L1; down-regulation: miR-1265	circMMP9/miR-1265/CHI3L1	[23]
Circ001621	Up	Promotes the proliferation and migration, higher TNM stage, shorter overall survival	Upregulation: VEGF/CDK4/MMP9; down-regulation: miR-578	mir-578/VEGF	[25]
Circ0001658	Up	Promotes the proliferation, migration, and invasion, hinder apoptosis	Upregulation: YB-1; down-regulation: mir-382-5p	circ0001658/miR-382-5p/YB-1	[26]
CircEPSTI1	Up	Cell proliferation and migration	Upregulation: MCL1; down-regulation: miR-892b	circEPSTI1-miR-892b-MCL1	[27]
CircANKIB1	Up	Cell proliferation, invasion, and inhibit apoptosis	Upregulation: miR-19b; down-regulation: SOCS3	circANKIB1/miR-19b/SOCS3	[28]
Circ-LARP4	Down	Decreased Enneking stage, prolonged survival profiles, and elevated chemosensitivity to cisplatin and doxorubicin	Downregulation: mir-424	_	[29]
Circ0002052	Down	Suppresses proliferation, migration and invasion while promoting apoptosis	Upregulation: APC2; down-regulation: miR-1205	circ0002052/miR-1205/APC2/Wnt/b-catenin	[30]
CircROCK1-E3/E4	Down	Suppresses proliferation and migration ability	Upregulation: PTEN; down-regulation: miR-532-5p	circROCK1-E3/E4/miR-532-5p/PTEN	[31]
CircNRIP1	Down	Suppresses migration, invasion and proliferation	Upregulation: MIA2; down-regulation: miR-1200	circNRIP1/miR-1200/MIA2	[32]
CircITCH (multifunctional)	Up	Promotes the migration, invasion, and growth	Upregulation: EGFR; down-regulation: miR-7	circITCH /miR-7/EGFR	[36]
	Down	Suppresses cell growth, migration, and invasion capacity promotes apoptosis	Upregulation: PTEN; down-regulation: miR-22	PTEN/PI3K/AKT and SP-1	[37]

invasion. At the same time, overexpression of circTCF25 can reduce the level of miR-206 in osteosarcoma cells. In this regard, MEK/ERK and AKT/mTOR pathways were identified as downstream pathways inhibited by miR-206.[21]

CircMMP9

Studies have shown that circMMP9 promotes the occurrence and development of glioblastoma.[22] Pan et al[23] found that the expression level of circMMP9 in osteosarcoma was high, which may lead to the rapid development of tumor and is related to TNM staging. TNM (tumor–node–metastasis) staging system codes the extent of the primary tumor (T), regional lymph nodes (N), and distant metastases (M) and provides a "stage grouping" based on T, N, and M.[24] The overall survival rate of osteosarcoma patients with high expression of circMMP9 was also lower than that of patients with low expression of circMMP9. Functionally, knockout of circMMP9 decreased the viability, colony-forming ability, migration, and invasion ability of osteosarcoma cells, suggesting that circMMP9 has a carcinogenic effect. It was found that the carcinogenesis of circMMP9 was mediated by sponging miR-1265 and decreased subsequent inhibition of CHI3L1 (chitinase-3-like protein 1). MiR-1265 was directly bound to the 3′ UTR of CHI3L1 mRNA and inhibited its expression. CHI3L1 levels were regulated by circMMP9/miR-1265 axis.

Circ001621

Circ001621 was identified as an upregulated circRNA in osteosarcoma by PCR array. Its high expression was associated with a higher TNM stage and shorter overall survival.[25] In this regard, miR-578 was the target of circ001621 in osteosarcoma, and their expression showed a strong negative correlation. Functional experiments and mechanism studies showed that circ001621 promoted the proliferation and migration of osteosarcoma cells in vitro by inhibiting miR-578 mediated VEGF inhibition and CDK4 and MMP9 expression. Significantly, circ001621 enhances the metastatic ability of osteosarcoma to the lung and liver and upregulates VEGF, CDK4, and MMP9.[25] In conclusion, circ001621 is a carcinogenic circular RNA that promotes osteosarcoma metastasis by regulating the miR-578/VEGF pathway.

Circ0001658

Circ0001658 was found to be upregulated in osteosarcoma compared with normal bone tissue. Functional experiments showed that circ0001658 promoted the proliferation, migration, and invasion of osteosarcoma cells and hindered apoptosis. These tumor-promoting effects are mediated by sponging miR-382-5p and increasing the expression of YB-1. There was a negative expression correlation between circ0001658 and miR-382-5p as well as miR-382-5p and YB-1. The expression of circ0001658 was positively correlated with YB-1. In conclusion, circ0001658 is a circular RNA that can promote cancer by regulating the miR-382-5p /YB-1 axis.[26]

CircEPSTI1

Tan et al[27] reported that circEPSTI1 was significantly upregulated in osteosarcoma. Knockout of circRNA inhibits cell proliferation and migration, suggesting its carcinogenic effect. CircEPSTI1 upregulates the expression of MCL1 by reducing the availability of miR892b. These data suggest that the circEPSTI1-miR-892b- MCL1 axis plays a vital role in osteosarcoma progression.

CircANKIB1

Du et al[28] showed that circANKIB1 increased miR-19b expression in osteosarcoma cells, enhanced the expression of circANKIB1-miR-19b, and inhibited the expression of the downstream target gene SOCS3. After knockout of circANKIB1-miR-19b, the body inhibits cell invasion and growth by regulating the STAT3 Pathway, thus inducing tumor cell apoptosis. These data suggest that circANKIB1 is an oncogene and a potential therapeutic target for osteosarcoma.

Carcinostatic Circular RNA

CircLARP4

Hu et al[29] showed that the level of circLARP4 in osteosarcoma tissue was lower than that in non-tumor tissue, and circLARP4 was related to the Enneking stage. In patients with high circLARP4 levels, the necrosis rate of tumor cells increased after resection and chemotherapy. High expression of circLARP4 was associated with prolonged overall

survival and disease-free survival. Overexpression of circLARP4 enhanced the chemosensitivity of MG63 cells to adriamycin and cisplatin but did not affect the chemosensitivity of MG63 cells to methionine. In addition, overexpression of miR-424 can reduce the expression of circLARP4, thus affecting the chemosensitivity and therapeutic effect of MG63 cells. Their data suggest that overexpression of circLARP4 shortens the Enneking phase by sponging miR-424, down-regulate the expression of miR-424, and overexpression of circLARP4 can enhance the chemosensitivity of cells to adriamycin and cisplatin, thereby interfering with the occurrence and development of osteosarcoma.

Circ0002052

Wu et al[30] showed that overexpression of Hsa_circ_0002052 significantly inhibited the proliferation, migration and invasion of OS cells, and promoted apoptosis in vitro. Hsa_circ_0002052 inhibited Mir-1205, which targeted APC2, a negative regulator of Wnt/b-catenin signaling pathway. In this study, hsa_circ_0002052 was identified as a mir-1205 sponge to facilitate APC2 expression and consequently inactivate Wnt/b-catenin signaling pathway, leading to reduced OS progression. The Hsa_circ_0002052/ Mir-1205/APC2/Wnt/b-catenin axis may be a potential target for OS treatment.

CircROCK1-E3/E4

The study of Liu et al[31] showed that circROCK1-E3/E4, produced from exons 3 and 4 of ROCK1 precursor mRNA, was downregulated in patients with osteosarcoma and associated with poor prognosis. Downregulation of circROCK1-E3/E4 promoted proliferation and migration in U2OS and HOS cells. In contrast, overexpression of circROCK1-E3/E4 suppressed the proliferation and migration ability. In U2OS and HOS cells, CircROCK1-E3/E4 sponges miR-532-5p which promotes proliferation and migration by targeting PTEN. Up-and downregulation of miR-532-5p inversely regulated PTEN expression. It was revealed that miR-532-5p was upregulated in 20 osteosarcoma plasma samples by using 15 healthy controls.

CircNRIP1

Hei et al[32] showed that circNRIP1 was down-regulated in OS cell lines. CircNRIP1 expression could significantly suppress OS cell proliferation, migration and invasion by sponging miR-1200 in vitro and in vivo. To investigate the in vivo function of circNRIP1 in OS cell xenograft growth, they established and evaluated a xenograft model of OS cells in nude mice. Increased MIA2 expression was observed in tissues from mice overexpressed circNRIP1. CircNRIP1 acts as a miRNA sponge by interacting with miR-1200 and its target gene MIA2, leading to upregulation of MIA2 expression and ultimately inhibition of osteosarcoma progression. CircNRIP1 inhibits OS progression through the miR-1200 /MIA2 axis.

CircITCH, a Multifunctional CircRNA

In esophageal squamous cell carcinoma, circITCH was found as a tumor suppressor RNA, and it was also found in[33] colorectal cancer[34] and lung cancer,[35] but its inhibitory effect in these cancer types was different. Li et al[36] reported that the expression level of circITCH in osteosarcoma cell lines was significantly higher than in hfob1.19 normal osteoblasts. Notably, the higher expression of circITCH in osteosarcoma promotes the proliferation, migration, and invasion of osteosarcoma cells, while the knockout of circITCH has the opposite effect. Current studies on osteosarcoma emphasize the regulation of different signaling pathways by the circRNA network. Therefore, erlotinib (an EGFR tyrosine kinase inhibitor) can eliminate the role of circITCH in promoting tumor growth and enhancing tumor invasion.[36] Ren et al[37] also found that circITCH can inhibit osteosarcoma progression by down-regulating miR-22. circITCH may function through PTEN/PI3K/AKT and SP-1pathways. PTEN negatively regulates PI3K/AKT pathway. PTEN expression was significantly escalated when overexpressing circ-ITCH and declined via upregulating miR-22.

Application of circRNA in the Diagnosis and Treatment of Osteosarcoma

With the development of science and medical technology, many tumors are no longer incurable, and the survival time of patients and quality of life have been significantly improved. For the treatment of osteosarcoma, surgery and neoadjuvant chemotherapy are mainly used, but the 5-year survival rate of patients still needs to be improved. Xu et al[38] found no survival advantage between preoperative chemotherapy and immediate surgery for a specific group of non-metastatic

high-grade pelvic osteosarcoma patients with an immense tumor burden and an inadequate response to chemotherapy. At 5 years, the overall survival rate was 43% in the neoadjuvant group and 40% in the immediate surgery group, with no significant difference. It is necessary to seek new therapeutic methods. Recent studies have shown that circRNA can be used as a target for tumor target therapy, and the expression of some circRNAs is significantly correlated with the grade of osteosarcoma and the survival time of patients[39] (Figure 1).

Diagnosis and Prognosis Analysis

CircRNA can stably exist in large quantities in various body fluids, including plasma, serum, exosomes, and urine.[40] The expression of circRNA was significantly different between tumor patients and healthy controls, which indicated that circRNA in body fluid could be used as a new biomarker for monitoring tumor development.[41] Zhang et al[42] showed that circRNA UBAP2 was overexpressed in OS, and its expression level was significantly correlated with the differentiation degree and prognosis of OS. The detection of circRNA UBAP2 content in serum exosomes can help the early diagnosis of osteosarcoma. The study of Long et al[43] showed that compared with normal adjacent tissues, circ001569, circCDRLAS, circHIPK3 and circfoxo3 were significantly down-regulated in osteosarcoma, among which circHIPK3 was significantly correlated with Enneking stage, lung cancer metastasis, and survival time, suggesting that the lower the expression of circHIPK3, the shorter the survival time and the worse the prognosis. Many studies have shown that the expression level of circRNAs can be used for early diagnosis, classification, staging, and prognosis analysis of osteosarcoma. Compared with invasive tests such as needle biopsy, the development of tumor markers diagnostic kit for circRNAs in body fluid is expected to make tumor diagnosis safer and more portable, which is also of great significance for early tumor screening (Figure 1).

Drug Resistance

Zhu et al[44] showed that circRNA PVT1 was screened to determine its carcinogenic effect on OS. circPVT1 was significantly upregulated in OS tissues, serum, and chemoresistant cell lines, which was related to the poor prognosis of OS patients. ROC curve showed that circPVT1 might be a better biomarker than alkaline phosphatase (ALP), with higher sensitivity and specificity. In addition, functional analysis showed that siRNA knockdown of circPVT1 could reduce the resistance of OS cells to adriamycin and cisplatin by reducing the expression of ABCB1. This may provide new insights into the role of circpvt1 as a biomarker for the diagnosis and treatment of OS. Gu et al[45] showed that has_circ_0010220 increased DOX-resistance in OS by miR-574-3p/IL-6 axis regulation. Reducing DOX resistance in vitro was achieved by silencing Has_circ_0010220. It suggested potency of circRNA as a promising biomarker for treating OS.

RNA Vaccine

Researchers from Tsinghua University show that[46] circRNA vaccine platform provides a novel prospect for the development of cancer RNA vaccines in a wide range of hard-to-treat malignancies. CircRNA can elongate the production of tumor antigen, thus prolonging the antigen presentation of antigen-presenting cells. The purified circRNA has low immunogenicity and cannot provide an inflammatory microenvironment for the activation of cytotoxic T cells. To solve this problem, they cooperated with a new type of ionizable lipid, which can induce the release of proinflammatory cytokines into LNP carrier. This binding exerts the long-term protein translation ability of circRNA, and provides an inflammatory immune environment suitable for cytotoxic T cell activation.

CircRNA-Based Drug Delivery Systems

Nanoparticles can carry drugs and deliver them to therapeutic targets. Lipid-based nanoparticles (LNPs) can be used to target specific cells using endogenous or exogenous ligands by encapsulating circRNA.[47] Gold nanoparticle (AuNP), a well-studied non-viral vector with high stability, purity, and easy surface modification, has been used to deliver circRNAs plasmids.[48] Research showed AuNP delivery of circFoxo3 plasmid can promote tumor cell apoptosis and inhibits tumor growth.[49]

Nucleic Acid Aptamer

RNA aptamers and RNA aptamer-based devices can be genetically encoded and expressed in cells to probe and regulate cellular function.[50] Because aptamers can bind specific domains and conformations of proteins,[51] they can either inhibit proteins or modulate their function. Circular RNA can be used as RNA aptamer. Comparable to the levels of proteins and the most abundant endogenous small RNAs, circular RNAs are highly stable and achieve significantly high expression levels in cells.[52]

The study of Al-Sudani et al[50] proved that there are two types of aptamer selection, circular and linear. AC3, a circular aptamer, stood up as the optimal aptamer for inducing SIRT1 activity and exerting anticancer action. The biophysical interactions between AC3 and SIRT1 were described using a modified library. Confirmed by co-localisation, AC3 was recruited to intracellular SIRT1. AC3 has shown anticancer activity against several cancer models including lung cancer, liver cancer and osteosarcoma. AC3 was non-toxic on the non-cancerous cell-line Beas2B, implying it might be safe for non-cancerous tissues.

Circular RNAs in Exosomes

The expression profiles of circRNA in the body fluids between osteosarcoma patients and healthy people are different,[53] such as circRNAs in exosomes. Exosomal circRNAs can be taken up by neighboring or distant cells and influence physiological and pathological status of the recipient cells.[54] The study of Pan et al[55] showed that hsa_circ_103801 was highly enriched in CDDP-resistant OS cell-derived exosomes. CDDP-resistant cell-derived exosomes could transfer hsa_circ_103801 to sensitive cells and enhance the resistance of OS cells. The survival time of OS patients with high expression of hsa_circRNA_103801 was shorter. These findings suggest that reducing hsa_circ_103801 expression in CDDP-resistant cell-derived exosomes may be a potential strategy to overcome chemotherapy resistance to OS. The study of Dou et al[56] showed that OS patients had higher circ_0056285 levels in serum exosomes than those in healthy volunteers. ROC curve showed that the level of exosomal hsa_circ_0056285 had high diagnostic value for OS. Circ_0056285 promoted OS progression by sponging miR-1244 and increasing TRIM44 expression. Functional experiments showed that circ_0056285 silencing inhibited the progression of OS cells by inhibiting cell proliferation and glycolysis and inducing cell apoptosis. Circ_0056285 knockdown blocked tumor growth in nude mice. It provided a promising therapeutic target for OS.

Exosomes represent a new way of intercellular communication, rich in circRNAs.[57] Many studies have shown that exosomal circRNA is involved in cell–cell communication in the tumor microenvironment and regulates many characteristics of tumors, such as proliferation, invasion and metastasis, angiogenesis, immune escape, and drug resistance.[58] In addition, exosomal circRNA carries the biological information related to the primary tumor, easily crosses various biological barriers, and exists in a large number of body fluids from different sources, which brings great hope for the diagnosis and treatment of osteosarcoma and can be used as a biomarker for early diagnosis and prognosis evaluation.[59] The isolation and detection of circRNA from tumor-associated exosomes need cutting-edge technology, so it is necessary to develop more accurate and straightforward detection methods to monitor and find new therapeutic targets.

Tumor-derived exosomes of nanometer size are easy to pass through biological barriers such as the blood–brain barrier.[60] They have natural homing characteristics, stability of bilayer lipid structure, and high biocompatibility. Therefore, tumor exosomes are becoming an ideal carrier for tumor target therapy; especially the engineered exosomes show the excellent effect of tumor-targeted intervention therapy. The application prospect of tumor exosomes in circRNA-related target therapy is worth looking forward to and further exploring.

Conclusion

CircRNA plays a vital role in various diseases, participates in the physiological and pathological regulation process, and can be used as a promising biomarker in disease detection and treatment.[61,62] But at present, as a cancer biomarker, specific circRNA is required to be differentially expressed after transformation or in the early stage of specific cancer and needs the support of related quantitative technology. This technology still needs to be studied for clinical application. It will be fruitful work to study the role of circRNA in cancer. Using circRNA as a cancer biomarker can be used for early cancer screening and increase the survival rate.

Understanding the role of circRNA will change and supplement the traditional understanding of the occurrence and development of osteosarcoma and help understand the molecular mechanism related to the occurrence and development

of osteosarcoma. CircRNA is widely involved in gene expression regulation and protein translation[63] and plays an indispensable role in the occurrence and development of osteosarcoma.[64] Liquid biopsy, circRNA-based prognosis analysis, RNA vaccine, circRNA-based drug delivery systems, reduction of circRNA-related drug resistance, application of nuclear acid aptamer, application of engineered exosome, etc. will benefit the individualized diagnosis and treatment of osteosarcoma in the future.

Abbreviations

circRNA, circular RNA; ecircRNA, exonic circRNA; EIciRNA, exon-intron circRNA; ciRNA, circular intronic RNA; tricRNA, tRNA intronic circRNA; RBP, RNA binding protein; ORF, open reading frame; OS, osteosarcoma; CHI3L1, chitinase-3-like protein 1; ALP, alkaline phosphatase.

Acknowledgments

The authors would like to thank Innovative Research and Development Platform for Individualized Precise Clinical Diagnosis and Treatment of Innovation and Entrepreneurship Incubation Base of China Medical University, for its support. The authors would like to thank Zesi Liu, Yuze Hu, Yifan Xiao, Linbo Zhang, Mohammad Showkat Hossain from China Medical University for their help and contributions.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was supported by the National Natural Science Foundation of China(81272946); The College Students' Innovation Project of China Medical University; Fujian Provincial Clinical Medical Research Center for First Aid and Rehabilitation in Orthopaedic Trauma(2020Y2014).

Disclosure

The authors declare that they have no competing interests.

References

1. Sanger H, Klotz G, Riesner D, Gross H, Kleinschmidt A. Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures. *Proc Natl Acad Sci*. 1976;73(11):3852–3856. doi:10.1073/pnas.73.11.3852
2. Arnberg A, Van Ommen G, Grivell L, Van Bruggen E, Borst P. Some yeast mitochondrial RNAs are circular. *Cell*. 1980;19(2):313–319. doi:10.1016/0092-8674(80)90505-X
3. Kos A, Dijkema R, Arnberg A, van der Meide P, Schellekens H. The hepatitis delta (delta) virus possesses a circular RNA. *Nature*. 1986;323 (6088):558–560. doi:10.1038/323558a0
4. Jeck W, Sorrentino J, Wang K, et al. Circular RNAs are abundant, conserved, and associated with ALU repeats. *RNA*. 2013;19(2):141–157. doi:10.1261/rna.035667.112
5. Qu S, Yang X, Li X, et al. Circular RNA: a new star of noncoding RNAs. *Cancer Lett*. 2015;365(2):141–148. doi:10.1016/j.canlet.2015.06.003
6. Schmidt C, Noto J, Filonov G, Matera A. A method for expressing and imaging abundant, stable, circular RNAs in vivo using tRNA splicing. *Methods Enzymol*. 2016;572:215–236.
7. Xia S, Feng J, Lei L, et al. Comprehensive characterization of tissue-specific circular RNAs in the human and mouse genomes. *Brief Bioinform*. 2017;18(6):984–992. doi:10.1093/bib/bbw081
8. Wang M, Xie F, Lin J, et al. Diagnostic and prognostic value of circulating CircRNAs in cancer. *Front Med*. 2021;8:649383. doi:10.3389/fmed.2021.649383
9. Panda A, De S, Grammatikakis I, et al. High-purity circular RNA isolation method (RPAD) reveals vast collection of intronic circRNAs. *Nucleic Acids Res*. 2017;45(12):e116. doi:10.1093/nar/gkx297
10. Saunders G, Baudis M, Becker R, et al. Leveraging European infrastructures to access 1 million human genomes by 2022. *Nat Rev Genet*. 2019;20 (11):693–701. doi:10.1038/s41576-019-0156-9
11. Rybak-Wolf A, Stottmeister C, Glažar P, et al. Circular RNAs in the mammalian brain are highly abundant, conserved, and dynamically expressed. *Mol Cell*. 2015;58(5):870–885. doi:10.1016/j.molcel.2015.03.027

12. Ashwal-Fluss R, Meyer M, Pamudurti N, et al. circRNA biogenesis competes with pre-mRNA splicing. *Mol Cell*. 2014;56(1):55–66. doi:10.1016/j.molcel.2014.08.019
13. Ng W, Mohd Mohidin T, Shukla K. Functional role of circular RNAs in cancer development and progression. *RNA Biol*. 2018;15(8):995–1005. doi:10.1080/15476286.2018.1486659
14. Wang Z, Lei X. Identifying the sequence specificities of circRNA-binding proteins based on a capsule network architecture. *BMC Bioinform*. 2021;22(1):19. doi:10.1186/s12859-020-03942-3
15. Du W, Yang W, Liu E, Yang Z, Dhaliwal P, Yang B. Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2. *Nucleic Acids Res*. 2016;44(6):2846–2858. doi:10.1093/nar/gkw027
16. Dong R, Zhang X, Zhang Y, Ma X, Chen L, Yang L. CircRNA-derived pseudogenes. *Cell Res*. 2016;26(6):747–750. doi:10.1038/cr.2016.42
17. Liu J, Liu T, Wang X, He A. Circles reshaping the RNA world: from waste to treasure. *Mol Cancer*. 2017;16(1):58. doi:10.1186/s12943-017-0630-y
18. Memczak S, Jens M, Elefsinioti A, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature*. 2013;495(7441):333–338. doi:10.1038/nature11928
19. Yan T, Wunder J, Gokgoz N, et al. COPS3 amplification and clinical outcome in osteosarcoma. *Cancer*. 2007;109(9):1870–1876. doi:10.1002/cncr.22595
20. Zhong Z, Lv M, Chen J. Screening differential circular RNA expression profiles reveals the regulatory role of circTCF25-miR-103a-3p/miR-107-CDK6 pathway in bladder carcinoma. *Sci Rep*. 2016;6:30919. doi:10.1038/srep30919
21. Wang Y, Shi S, Zhang Q, Dong H, Zhang J. MicroRNA-206 upregulation relieves circTCF25-induced osteosarcoma cell proliferation and migration. *J Cell Physiol*. 2020;2020:114.
22. Wang R, Zhang S, Chen X, et al. EIF4A3-induced circular RNA MMP9 (circMMP9) acts as a sponge of miR-124 and promotes glioblastoma multiforme cell tumorigenesis. *Mol Cancer*. 2018;17(1):166. doi:10.1186/s12943-018-0911-0
23. Pan G, Hu T, Chen X, Zhang C. Upregulation of circMMP9 promotes osteosarcoma progression via targeting miR-1265/CHI3L1 axis. *Cancer Manag Res*. 2019;11:9225–9231. doi:10.2147/CMAR.S226264
24. Edge S, Compton C. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol*. 2010;17(6):1471–1474. doi:10.1245/s10434-010-0985-4
25. Ji X, Shan L, Shen P, He M. Circular RNA circ_001621 promotes osteosarcoma cells proliferation and migration by sponging miR-578 and regulating VEGF expression. *Cell Death Dis*. 2020;11(1):18. doi:10.1038/s41419-019-2204-y
26. Wang L, Wang P, Su X, Zhao B. Circ_0001658 promotes the proliferation and metastasis of osteosarcoma cells via regulating miR-382-5p/YB-1 axis. *Cell Biochem Funct*. 2020;38(1):77–86. doi:10.1002/cbf.3452
27. Tan X, Tan D, Li H, Lin Y, Wen Z, Zeng C. circEPSTI1 acts as a ceRNA to regulate the progression of osteosarcoma. *Curr Cancer Drug Targets*. 2020;20(4):288–294. doi:10.2174/1568009619666191107140948
28. Du Y, Guo L, Pan H, Liang Y, Li X. Circ_ANKIB1 stabilizes the regulation of miR-19b on SOCS3/STAT3 pathway to promote osteosarcoma cell growth and invasion. *Hum Cell*. 2020;33(1):252–260. doi:10.1007/s13577-019-00298-6
29. Hu Y, Gu J, Shen H, et al. Circular RNA LARP4 correlates with decreased Enneking stage, better histological response, and prolonged survival profiles, and it elevates chemosensitivity to cisplatin and doxorubicin via sponging microRNA-424 in osteosarcoma. *J Clin Lab Anal*. 2020;34(2):e23045. doi:10.1002/jcla.23045
30. Wu Z, Shi W, Jiang C. Overexpressing circular RNA hsa_circ_0002052 impairs osteosarcoma progression via inhibiting Wnt/β-catenin pathway by regulating miR-1205/APC2 axis. *Biochem Biophys Res Commun*. 2018;502(4):465–471. doi:10.1016/j.bbrc.2018.05.184
31. Liu Y, Qiu G, Luo Y, et al. Circular RNA ROCK1, a novel circRNA, suppresses osteosarcoma proliferation and migration via altering the miR-532-5p/PTEN axis. *Exp Mol Med*. 2022;54(7):1024–1037. doi:10.1038/s12276-022-00806-z
32. Hei R, Chen J, Zhang X, et al. CircNRIP1 acts as a sponge of miR-1200 to suppress osteosarcoma progression via upregulation of MIA2. *Am J Cancer Res*. 2022;12(6):2833–2849.
33. Li F, Zhang L, Li W, et al. Circular RNA ITCH has inhibitory effect on ESCC by suppressing the Wnt/β-catenin pathway. *Oncotarget*. 2015;6(8):6001–6013. doi:10.18632/oncotarget.3469
34. Huang G, Zhu H, Shi Y, Wu W, Cai H, Chen X. cir-ITCH plays an inhibitory role in colorectal cancer by regulating the Wnt/β-catenin pathway. *PLoS One*. 2015;10(6):e0131225. doi:10.1371/journal.pone.0131225
35. Wan L, Zhang L, Fan K, Cheng Z, Sun Q, Wang J. Circular RNA-ITCH suppresses lung cancer proliferation via inhibiting the Wnt/β-catenin pathway. *Biomed Res Int*. 2016;2016:1579490. doi:10.1155/2016/1579490
36. Li H, Lan M, Liao X, Tang Z, Yang C. cir-ITCHCircular RNA promotes osteosarcoma migration and invasion through /miR-7/EGFR pathway. *Technol Cancer Res Treat*. 2020;19:1533033819898728.
37. Ren C, Liu J, Zheng B, Yan P, Sun Y, Yue B. The circular RNA circ-ITCH acts as a tumour suppressor in osteosarcoma via regulating miR-22. *Artif Cells*. 2019;47(1):3359–3367. doi:10.1080/21691401.2019.1649273
38. Xu J, Xie L, Guo W. Neoadjuvant chemotherapy followed by delayed surgery: is it necessary for all patients with nonmetastatic high-grade pelvic osteosarcoma? *Clin Orthop Relat Res*. 2018;476(11):2177–2186. doi:10.1097/CORR.0000000000000387
39. Zhang C, He J, Qi L, et al. Diagnostic and prognostic significance of dysregulated expression of circular RNAs in osteosarcoma. *Expert Rev Mol Diagn*. 2021;21(2):235–244. doi:10.1080/14737159.2021.1874922
40. Zhang Z, Yang T, Xiao J. Circular RNAs: promising biomarkers for human diseases. *EBioMedicine*. 2018;34:267–274. doi:10.1016/j.ebiom.2018.07.036
41. Wang S, Zhang K, Tan S, et al. Circular RNAs in body fluids as cancer biomarkers: the new frontier of liquid biopsies. *Mol Cancer*. 2021;20(1):13. doi:10.1186/s12943-020-01298-z
42. Zhang H, Wang G, Ding C, et al. Increased circular RNA UBAP2 acts as a sponge of miR-143 to promote osteosarcoma progression. *Oncotarget*. 2017;8(37):61687–61697. doi:10.18632/oncotarget.18671
43. Xiao-Long M, Kun-Peng Z, Chun-Lin Z. Circular RNA circ_HIPK3 is down-regulated and suppresses cell proliferation, migration and invasion in osteosarcoma. *J Cancer*. 2018;9(10):1856–1862. doi:10.7150/jca.24619
44. Kun-Peng Z, Xiao-Long M, Chun-Lin Z. Overexpressed circPVT1, a potential new circular RNA biomarker, contributes to doxorubicin and cisplatin resistance of osteosarcoma cells by regulating ABCB1. *Int J Biol Sci*. 2018;14(3):321–330. doi:10.7150/ijbs.24360
45. Gu Y, Wang G, Ran B. Has_circ_0010220 regulates the miR-574-3p/IL-6 axis to increase doxorubicin resistance in osteosarcoma. *Hum Exp Toxicol*. 2022;41:9603271221131307. doi:10.1177/09603271221131307

46. Li H, Peng K, Yang K, et al. Circular RNA cancer vaccines drive immunity in hard-to-treat malignancies. *Theranostics*. 2022;12(14):6422–6436. doi:10.7150/thno.77350
47. Zhao X, Zhong Y, Wang X, Shen J, An W. Advances in circular RNA and its applications. *Int J Med Sci*. 2022;19(6):975–985. doi:10.7150/ijms.71840
48. Ojea-Jiménez I, Comenge J, García-Fernández L, Megson Z, Casals E, Puntes V. Engineered inorganic nanoparticles for drug delivery applications. *Curr Drug Metab*. 2013;14(5):518–530. doi:10.2174/13892002113149990008
49. Du W, Fang L, Yang W, et al. Induction of tumor apoptosis through a circular RNA enhancing Foxo3 activity. *Cell Death Differ*. 2017;24(2):357–370. doi:10.1038/cdd.2016.133
50. Al-Sudani B, Ragazzon-Smith A, Aziz A, et al. Circular and linear: a tale of aptamer selection for the activation of SIRT1 to induce death in cancer cells. *RSC Adv*. 2020;10(73):45008–45018. doi:10.1039/D0RA07857C
51. Umekage S, Kikuchi Y. In vitro and in vivo production and purification of circular RNA aptamer. *J Biotechnol*. 2009;139(4):265–272. doi:10.1016/j.jbiotec.2008.12.012
52. Litke J, Jaffrey S. Highly efficient expression of circular RNA aptamers in cells using autocatalytic transcripts. *Nat Biotechnol*. 2019;37(6):667–675. doi:10.1038/s41587-019-0090-6
53. Chen Z, Li L, Li Z, et al. Identification of key serum biomarkers for the diagnosis and metastatic prediction of osteosarcoma by analysis of immune cell infiltration. *Cancer Cell Int*. 2022;22(1):78. doi:10.1186/s12935-022-02500-6
54. Shi X, Wang B, Feng X, Xu Y, Lu K, Sun M. circRNAs and exosomes: a mysterious frontier for human cancer. *Mol Ther Nucleic Acids*. 2020;19:384–392. doi:10.1016/j.omtn.2019.11.023
55. Pan Y, Lin Y, Mi C. Cisplatin-resistant osteosarcoma cell-derived exosomes confer cisplatin resistance to recipient cells in an exosomal circ_103801-dependent manner. *Cell Biol Int*. 2021;45(4):858–868. doi:10.1002/cbin.11532
56. Huo S, Dou D. Circ_0056285 regulates proliferation, apoptosis and glycolysis of osteosarcoma cells via miR-1244/TRIM44 axis. *Cancer Manag Res*. 2021;13:1257–1270. doi:10.2147/CMAR.S290645
57. Han Z, Chen H, Guo Z, et al. Circular RNAs and their role in exosomes. *Front Oncol*. 2022;12:848341. doi:10.3389/fonc.2022.848341
58. Ye D, Gong M, Deng Y, et al. Roles and clinical application of exosomal circRNAs in the diagnosis and treatment of malignant tumors. *J Transl Med*. 2022;20(1):161. doi:10.1186/s12967-022-03367-x
59. Verdi J, Ketabchi N, Noorbakhsh N, et al. Development and clinical application of tumor-derived exosomes in patients with cancer. *Curr Stem Cell Res Ther*. 2022;17(1):91–102. doi:10.2174/1574888X16666210622123942
60. Farooqi A, Desai N, Qureshi M, et al. Exosome biogenesis, bioactivities and functions as new delivery systems of natural compounds. *Biotechnol Adv*. 2018;36(1):328–334. doi:10.1016/j.biotechadv.2017.12.010
61. Wen G, Zhou T, Gu W. The potential of using blood circular RNA as liquid biopsy biomarker for human diseases. *Protein Cell*. 2021;12(12):911–946.
62. Han B, Chao J, Yao H. Circular RNA and its mechanisms in disease: from the bench to the clinic. *Pharmacol Ther*. 2018;187:31–44. doi:10.1016/j.pharmthera.2018.01.010
63. Pamudurti N, Bartok O, Jens M, et al. Translation of CircRNAs. *Mol Cell*. 2017;66(1):9–21.e7. doi:10.1016/j.molcel.2017.02.021
64. Liu J, Yang L, Fu Q, Liu S. Emerging roles and potential biological value of CircRNA in osteosarcoma. *Front Oncol*. 2020;10:552236. doi:10.3389/fonc.2020.552236