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Emerging Roles of Circular RNAs in Osteosarcoma

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Osteosarcoma (OS) is a primary malignant bone tumor in early adolescence with high metastasis and death rates. Although the combination of polychemotherapy and surgical excision increased the survival rates up to 60%, the prognosis remains poor for most patients with metastatic or recurrent osteosarcoma. However, the exact pathogenic mechanism and pivotal elements regulating tumor invasion and metastasis are largely unknown. Circular RNAs (circRNAs) are novel endogenous non-coding RNA (ncRNA) molecules that generate the cyclic structure from back splicing. An increasing number of studies show that circRNAs can regulate transcriptional or posttranscriptional gene expression by acting as microRNA (miRNA) sponges and are involved in regulation of many important biological processes. The deregulation of some circRNAs was demonstrated in osteosarcoma. Furthermore, some circRNAs were identified to play essential roles in osteosarcoma occurrence, invasion, and metastasis. This review summarizes the regulatory effect of circRNAs in the occurrence and development of osteosarcoma, concentrating on deregulation, regulatory mechanisms, and functions of circRNAs and their potential value as biomarkers and therapy.

MeSH Keywords: Biological Markers • Osteosarcoma • RNA, Circular • RNA, Untranslated

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Background

Osteosarcoma (OS) is the most common primary bone tumor of the skeleton, occurring mainly in children and adolescents [1]. Osteosarcoma primarily occurs in the metaphysis of long bones, mainly in the distal femur (43%), proximal tibia (23%), and humerus (10%) [2]. Before the 1970s, the treatment of osteosarcoma was relatively simple, with surgical resection of the tumor segment the only standard treatment method, and the long-term survival rate was less than 20% [3,4]. In recent years, the application of multiagent chemotherapy regimens significantly improved the prognosis for patients with unmetastasized osteosarcoma, and improved the long-term survival rate to 65–70% [5]. Approximately 15–20% of patients were found to have clinical metastasis at presentation, and their 5-year survival rate was less than 30% [6,7]. To improve the prognosis and survival rates of osteosarcoma patients, novel biomarkers and therapeutic targets are needed for early diagnosis and therapy of osteosarcoma.

Non-coding RNAs (ncRNAs) are a class of endogenous RNAs that participate in regulation of gene expression rather than being translated into proteins. In recent years, the regulatory effect of ncRNAs in multiple pathophysiological process have received extensive attention [8]. According to the transcript length, ncRNAs can be divided into 3 types: short ncRNAs (less than 50 nucleotides), mid-size ncRNAs (50–200 nucleotides), and long non-coding RNAs (lncRNAs, longer than 200 nucleotides) [9–11]. Short ncRNAs, named microRNAs (miRNAs, 18–24 nucleotides), can bind to the 3'-untranslated regions (3'-UTRs) of their target messenger RNAs (mRNAs) to promote mRNAs degradation and/or inhibit translation so as to inhibit target gene expression [12]. lncRNAs are versatile molecules that affect DNA, RNA, or protein to promote or restrain the expression of protein-coding genes [13]. In recent years, studies have demonstrated the regulatory effect of some miRNAs and lncRNAs in osteosarcoma.

Circular RNA (circRNA) is a new type of endogenous non-coding RNA, and has become a recent research hotspot in the field of molecular biology. Unlike the traditional linear RNAs, circRNAs are characterized by closed-loop structures without 5'-3' polarity or a polyadenylated tail, and their annular structure gives them strong resistance to RNA exonucleases or RNase R [14–17]. Currently, it is accepted that circRNAs act as miRNA sponges, regulating linear RNA transcription and protein production, and thereby regulating various pathophysiological processes [18,19]. To date, the deregulation of circRNAs has been studied in a series of biological processes and diseases, especially cancer occurrence and progression. In this article, we review the regulatory effect of circRNAs in osteosarcoma, concentrating on the progress of the deregulation, regulatory mechanisms, and functions (Table 1).

CircRNAs of Functional Significance in Osteosarcoma

Upregulated circRNAs in osteosarcoma

CircNASP

CircNASP, also known as hsa_circ_0092340, is significantly overexpressed in osteosarcoma cell lines compared with normal controls [20]. In osteosarcoma cell lines, knockdown of circNASP inhibits the G0/G1 stage of the cell cycle, inhibiting proliferation [20]. The Transwell assay result showed that circNASP knockdown significantly suppressed OS cell invasion [20]. miR-1253, a suppresser of cell proliferation and invasion in non-small-cell lung carcinoma by targeting FOXF1, was predicted to be associated with circNASP by bioinformatics analysis and luciferase reporter assay [20–31]. In MG63 and 143B cells, circNASP silencing promoted the expression level of miR-1253, which in turn inhibited the expression of FOXF1 at mRNA and protein levels, while activating miR-1253 had the opposite effect, demonstrating that circNASP exerts its roles in regulating FOXF1 by sponging miR-1253 in osteosarcoma cells [20]. Moreover, circNASP knockdown has inhibitory effects on osteosarcoma cell proliferation, cell cycle, and invasion, while inhibition of miR-1253 significantly reverses these effects [20]. FOXF1, identified as a target gene of miR-1253, was upregulated in osteosarcoma tissues, and maintaining FOXF1 at the normal level abolishes the inhibitory effects of miR-1253 overexpression on osteosarcoma cell proliferation, cell cycle, and invasion [20]. These data suggest that circNASP modulates cell proliferation, cell cycle, and tumor invasion by regulating miR-1253/FOXF1, and may serve as a novel therapeutic target of osteosarcoma.

CircPVT1

CircPVT1, derived from the oncogene PVT1 locus, is upregulated in several kinds of cancer tissues, including osteosarcoma, gastric cancer, acute lymphoblastic leukemia, and head and neck squamous cell carcinoma [21,32–34]. Higher expression of circPVT1 is associated with poor clinical prognosis in osteosarcoma patients [21]. As a diagnostic biomarker, circPVT1 is more reliable than ALP and is comparable to LDH [21]. Compared to the control, circPVT1 was significantly upregulated in osteosarcoma cell lines and chemoresistant osteosarcoma cell lines, suggesting the regulatory role of circPVT1 in the progression and chemoresistance of osteosarcoma [21]. Further *in vitro* studies revealed that silencing circPVT1 partly reversed the chemoresistance of osteosarcoma by reducing ABCB1, a classical multidrug resistance-related gene [21]. These results reveal that circPVT1 may serve as a novel diagnostic and therapeutic biomarker of osteosarcoma.

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Table 1. Functional characterization of the deregulated circRNAs in osteosarcoma.

No.	CircRNA	Alias	Deregulation	Gene symbol	Gene position	Functional role	Genes/proteins affected	Clinical value	Ref.
1	circNASP	hsa_circ_0092340	Up	NASP	chr1: 46079180-46079500	Proliferation(+); invasion and metastasis(+)	┤miR-1253, →FOXF1	Therapeutic	[20]
2	circPVT1	hsa_circ_0001821	Up	TCONS_00015354	chr8: 128902834-128903244	Chemoresistance(+); prognosis(–)	→ABCB1	Diagnostic, therapeutic	[21]
3	hsa_circ_0016347	–	Up	KCNH1	chr1: 211092981-211192598	Proliferation(+); invasion and metastasis(+)	┤miR-214, →caspase-1	Therapeutic	[22]
4	circNT5C2	hsa_circ_0092509	Up	NT5C2	chr10: 104850367-104850753	Proliferation(+); invasion(+); apoptosis(–)	┤miR-448	Therapeutic, diagnostic	[23]
5	hsa_circ_0001564	–	Up	CANX	chr5: 179132679-179137066	Proliferation(+); apoptosis(–); tumorigenicity(+)	┤miR-29c-3p	Therapeutic	[24]
6	hsa_circ_0009910	–	Up	MFN2	chr1: 12049221-12052747	Proliferation(+); apoptosis(–)	┤miR-449a, →IL6R, →JAK1/STAT3 signaling pathway	Therapeutic	[25]
7	circUBAP2	Not mentioned in the manuscript	Up	UBAP2	chr9	Proliferation(+); apoptosis(–)	┤miR-143, →Bcl-2	Therapeutic, prognostic	[26]
8	circGLI2	hsa_circ_0056288	Up	GLI2	chr2: 121708818-121713006	Proliferation(+); migration and invasion(+)	┤miR-125b-5p	Diagnostic, therapeutic	[27]
9	hsa_circ_0008717	–	Up	ABCB10	chr1: 229665945-229678118	Proliferation(+); migration and invasion(+); apoptosis(–)	┤miR-203, →Bmi-1	Therapeutic, prognostic	[28]
10	circHIPK3	hsa_circ_0000284	Down	HIPK3	chr11: 33307958-33309057	Proliferation(–); migration and invasion(–)	–	Diagnostic, therapeutic, prognostic	[29]
11	hsa_circ_0002052	–	Down	PAPPA	chr9: 118969734-118997916	Proliferation(–); migration and invasion(–); apoptosis(+)	┤miR-1205, →APC2, ┤Wnt/β-catenin signaling pathway	Therapeutic, prognostic	[30]

"┤" – inhibitory roles; "→" – stimulatory roles. circRNA – circular RNA; FOXF1 – forkhead box F1; ABCB1 – ATP binding cassette subfamily B member 1; IL6R – interleukin 6 receptor; Bcl-2 – B cell lymphoma 2; Bmi-1 – Bmi1 polycomb ring finger oncogene; APC2 – adenomatous polyposis coli 2; Ref – reference.

Hsa-circ-0016347

Hsa-circ-0016347, a circRNA assessed by bioinformatics analysis and later validated with qRT-PCR, is significantly overexpressed in osteosarcoma tissues and cell lines [22]. *In vitro* studies with CCK-8, wound-healing, and Transwell assays showed that circ-0016347 knockdown significantly reduces MG-63 and Saos-2 cell proliferation and invasion [22]. In further *in vivo* studies by subcutaneous injection and tail intravenous injection of transfected pcDNA-circ-0016347 OS cells in nude mice, the tumor sizes and the numbers of pulmonary metastasis tumors were distinctly increased in the circ-0016347-overexpressed mice, indicating that circ-0016347 plays a positive role in the proliferation, invasion, and metastasis of osteosarcoma cells [22]. Caspase-1, reported to contribute to the cell proliferation and invasion of osteosarcoma, was significantly increased in osteosarcoma tissues and cell lines, consistent with circ-0016347, and silencing circ-0016347 resulted in reduced

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caspase-1 expression [22]. Bioinformatics analysis and dual-luciferase reporter assay verified that caspase-1 is the direct target of miR-214 [22]. Further *in vitro* studies showed that the levels of miR-214 were significantly elevated in cells transfected with si-circRNA-0016347, indicating that circ-0016347 is negatively correlated with miR-214 and indirectly influences the expression of caspase-1 in osteosarcoma cells [22]. These data suggest that hsa-circ-0016347 sponges target miR-214 and is involved in regulating the proliferation and metastasis of osteosarcoma.

CircNT5C2

Circ-NT5C2 (hsa_circ_0092509) was screened out to be significantly overexpressed in osteosarcoma tissues by microarray and later validated with qRT-PCR [23]. Circ-NT5C2 showed a diagnostic potential, with an AUC (area under the curve) value of 0.753 in receiver operating characteristic (ROC) curve analysis [23]. Further *in vitro* studies with CCK-8 and colony formation assay showed that knockdown of circ-NT5C2 inhibited cell proliferation and increased the apoptotic cell rate of osteosarcoma compared to a negative control group [23]. An *in vivo* xenograft mouse study revealed that circ-NT5C2 knockdown reduced tumor volume and tumor weight, suggesting the suppressor role of circ-NT5C2 knockdown on osteosarcoma tumor growth [23]. Bioinformatics prediction and dual-luciferase reporter assay revealed that circ-NT5C2 sponged target miR-448, a miRNA reported to be involved in regulating the tumorigenesis and progression of multiple cancers including breast cancer, bladder cancer, pancreatic ductal adenocarcinoma, and osteosarcoma [23,35–38]. miR-448 expression levels were significantly downregulated in osteosarcoma tissue, and the expression of circ-NT5C2 and miR-448 showed a negative association [23]. CCK-8 assay and colony formation assay revealed that miR-448 inhibitor reverses the role of si-circ-NT5C2 on osteosarcoma cells (U2OS) proliferation, indicating that circ-NT5C2 sponges miR-448 [23]. In conclusion, circ-NT5C2 may act as a novel biomarker in osteosarcoma diagnosis and may participate in osteosarcoma progression.

Hsa_circ_0001564

Hsa_circ_0001564, located at 5q35.3, was originally selected as a significantly overexpressed circRNA in osteosarcoma tissue by microarray and later verified by qRT-PCR [24]. It was also significantly increased in osteosarcoma cell lines, especially HOS and MG-63 [24]. Function experiments revealed that hsa_circ_0001564 knockdown observably repressed the cell viability and colony formation ability of osteosarcoma cell lines, and the lower expression significantly induced cell cycle arrest in G0/G1 phase, indicating that hsa_circ_0001564 knockdown suppressed cells proliferation and promoted apoptosis and thus suppressed tumorigenicity of osteosarcoma cell lines [24]. miR-29c-3p, a downregulated miRNA in osteosarcoma tissue, was predicted to have binding sites with hsa_circ_0001564 by bioinformatics analysis and then confirmed by dual-luciferase reporter assay, indicating that hsa_circ_0001564 directly targets miR-29c-3p by acting as a sponge [24]. Further rescue experiments revealed that miR-29c-3p inhibition rescued the suppression, proliferation, and colony formation ability, and reversed the cell cycle arrest in G0/G1 phase and apoptosis induced by si-hsa_circ_001564 [24]. These data reveal the oncogenesis effect of hsa_circ_001564, and also illustrate the important role of competing endogenous RNAs mechanisms of hsa_circ_001564 and miR-29c-3p.

Hsa_circ_0009910

Hsa_circ_0009910, located at chr1: 12049221-12052747 with 315 bp length and gene symbol MFN2, was found to be overexpressed in osteosarcoma cell lines [25]. Knockdown of circ_0009910 induced cell proliferation inhibition, cell cycle arrest, and apoptosis in OS cell lines [25]. miR-449a, a downregulated miRNA in OS cells lines, was predicted as the target miRNA by bioinformatics analysis, and its expression level showed a negative correlation with that of circ_0009910 in OS tissues [25]. Dual-luciferase reporter assay confirmed the direct interaction of circ_0009910 and miR-449a [25]. Inhibition of miR-449a abrogates the effect of circ_0009910 knockdown on cell growth and apoptosis, suggesting the 'sponge' role of circ_0009910 in regulating miR-449a [25]. IL6R, whose mRNA expression is inversely correlated with miR-449a in OS tissues, was predicted to be a potential target of miR-449a [25]. Overexpression of miR-449a decreased the mRNA and protein levels of IL6R, and restoration of IL6R impaired the miR-449a-induced inhibition of cell proliferation, cell cycle arrest, and apoptosis [25]. Further signaling pathway research revealed that the JAK1/STAT3 pathway, reported to be associated with proliferation and apoptosis, is regulated by circ_0009910/miR-449a/IL6R axis [25]. These data indicate that hsa_circ_0009910 functions as a sponge of miR-449a, and may serve as a potential therapeutic target to reduce OS tumor growth.

CircUBAP2

CircRNA UBAP2 (circUBAP2) was originally screened by circRNA microarray to be markedly increased circRNA in osteosarcoma tissue as compared to adjacent normal tissues, then confirmed by qRT-PCR, and the deregulation status was also verified in osteosarcoma cell lines [26]. Spearman's rank correlation assay revealed that circUBAP2 expression was significantly positively correlated with tumor stages of osteosarcoma, and increased circUBAP2 expression in osteosarcoma tissues was significantly correlated with reduced survival and poor prognosis [26]. Function studies demonstrated that circUBAP2 overexpression promotes cell proliferation in osteosarcoma cell lines, whereas

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knockdown of circUBAP2 expression suppresses cell proliferation [26]. Further *in vivo* experiments showed that cell growth was significantly promoted in circUBAP2 stably-overexpressed osteosarcoma cell lines [26]. Bioinformatics analysis and RNA precipitation demonstrated that miR-143 is the circUBAP2-associated miRNA in osteosarcoma cells [26]. Previous research suggests that anti-apoptotic Bcl-2 is directly targeted by miR-143, and miR-143 is downregulated in osteosarcoma and causes the upregulation of anti-apoptotic Bcl-2 [39]. In human osteosarcoma tissues, circUBAP2 expression was found to be reversely correlated with miR-143 expression, confirming its sponge function to inhibit miR-143 expression [26]. CircUBAP2 could be used as a prognosis biomarker for poor survival of OS patients, and as a potential therapeutic target for inhibiting OS tumor growth.

CircGLI2

CircGLI2, also known as hsa_circ_0056288, is generated from pre-mRNA of GLI2, which is a primary transcriptional regulator mediating the activation of Hedgehog (Hh) signaling [27]. Compared with the negative control, circGLI2 was significantly overexpressed in both osteosarcoma tissue and cell lines [27]. Function studies revealed that knockdown of circGLI2 significantly suppressed cell proliferation and migration while increasing the apoptotic cell rate, suggesting the tumorigenic role of circGLI2 in osteosarcoma [27]. miR-125b-5p, which is downregulated in osteosarcoma tissues, was predicted to have negative regulatory relationships with circGLI2, and this was confirmed by dual-luciferase reporter assay [27]. Functional experiments revealed that miR-125b-5p overexpression inhibits cell proliferation, migration, and invasion, while silencing circGLI2 rescues the inhibitory effect, demonstrating the oncogenic role of circGLI2 by targeting miR-125b-5p [27]. These data suggest that circGLI2 is involved in regulating osteosarcoma progression by acting as a sponge of miR-125b-5p, and may serve as a potential target for osteosarcoma treatment.

Hsa_circ-0008717

Hsa_circ-0008717, a circRNA screened by microarray and later validated by qRT-PCR, was found to be significantly upregulated in osteosarcoma tissues compared with non-tumor tissue samples, and the upregulation was closely correlated with tumor metastasis and poor prognosis in osteosarcoma patients [28]. The same deregulation condition of hsa_circ-0008717 was also observed in osteosarcoma cell lines [28]. Knockdown of circRNA-0008717 significantly inhibited cell proliferation, colony formation ability, wound-healing ability, and invasive capabilities of osteosarcoma cells, while increasing the proportion of apoptotic cells [28]. Bioinformatics analysis and dual-luciferase reporter assay verified that miR-203 directly interacts with circRNA-0008717, and further RNA precipitation (RIP) result showed a specific enrichment of cirRNA-0008717 and miR-203, indicating that cirRNA-0008717 specifically interacts with miR-203 in osteosarcoma cells [28]. Further experiments demonstrated that miR-203 targets BMI-1 and suppresses osteosarcoma cell proliferation and invasion, and circRNA-0008717 regulates cell proliferation and invasion through sponging miR-203 and subsequently elevating Bmi-1 [28]. These data suggest that hsa_circ-0008717 regulates osteosarcoma tumor growth and metastasis by acting as a sponge of miR-124-3p, and may serve as a prognostic and therapeutic biomarker for osteosarcoma.

Downregulated circRNAs in osteosarcoma

CircHIPK3

CircHIPK3 (hsa_circ_0000284) is derived from exon2 of the HIPK3 gene, a serine-threonine kinase regulating transcription and apoptosis, and has been reported to regulate cellular processes by sponging multiple miRNAs [40,41]. CircHIPK3 has been reported to regulate cancer cell proliferation and metastasis by sponging miR-7, miR-124, and miR-558 [42–45]. In osteosarcoma cell lines, circHIPK3 was significantly downregulated, and it also had stable lower expression in osteosarcoma tissue and plasma [29]. Kaplan-Maier analysis showed that patients with lower expression of circHIPK3 had shorter overall survival time than those with higher circHIPK3 expression, and lung metastasis and advanced cancer were associated with lower expression levels of circHIPK3 [29]. The U2OS (or 143B) cells transfected with circHIPK3 showed lower cell proliferation rates and significantly suppressed cell proliferation ability, demonstrating that overexpression of circHIPK3 suppressed proliferation in OS cells [29]. Transwell and wound=healing assays showed that circHIPK3 overexpression significantly reduced cell migration [29]. These findings suggest that circ_HIPK3 is a potential diagnostic and prognostic biomarkers in OS, and may serve as a tumor-suppressor in osteosarcoma progression.

Hsa_circ_0002052

Hsa_circ_0002052 was found to be significantly downregulated in osteosarcoma cell lines in a circRNA microarray dataset (GSE96964), and qRT-PCR validated the microarray result [30]. The expression levels of hsa_circ_0002052 showed a significant negative correlation with survival and prognosis of osteosarcoma patients [30]. Functional studies revealed that overexpression of hsa_circ_0002052 obviously suppressed osteosarcoma cell proliferation and invasion and increased the apoptosis rate [30]. Bioinformatics prediction and dual-luciferase reporter assay verified that miR-1205 is a direct regulatory target of hsa_circ_0002052 [30]. Downstream, APC2, known as a negative regulatory factor of the Wnt/β-catenin pathway, was identified as a target of miR-1205 [30,46,47]. Further functional

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Table 2. The functional role of osteosarcomatous specifically expressed circRNAs in other diseases.

CircRNA	Disease type	Functional role	Genes/proteins affected	Expression	Ref.
circPVT1	Acute lymphoblastic leukemia	CircPVT1 promotes cell proliferation and inhibits apoptosis via targeting c-Myc and Bcl-2	→c-Myc, Bcl-2	Up	[49]
	Head and neck squamous cell carcinoma (HNSCC)	CircPVT1 is associated with TP53 mutations, and play the oncogenic role in HNSCC	┤miR-497-5p	Up	[34]
	Gastric cancer	CircPVT1 serves as a novel proliferative factor and prognostic marker in gastric cancer	┤miR-125	Up	[32]
circUBAP2	Lung cancer	CircUBAP2 promotes cancer cell proliferation and invasion of human lung cancer	┤miR-339-5p, miR-96-3p, miR-135b-3p	Up	[50]
circHIPK3	Nasopharyngeal carcinoma (NPC)	CircHIPK3 facilitates NPC progression through protecting ELF3 from miR-4288-mediated silencing	┤miR-4288, →ELF3	Up	[51]
	Glioma	CircHIPK3 contributes to glioma progression through targeting miR-654 from IGF2BP3	┤miR-654, →IGF2BP3	Up	[52]
	Epithelial ovarian cancer (EOC)	CircHIPK3 serves as a prognostic biomarker for EOC	-	Up	[53]
	Gallbladder cancer	CircHIPK3 promotes gallbladder cancer cell proliferation	┤miR-124, →ROCK1-CDK6	Up	[54]
	Colorectal cancer (CRC)	CircHIPK3 acts as a tumorigenic factor via the c-Myb/circHIPK3/miR-7 axis and may serve as a prognostic biomarker in CRC	┤miR-7	Up	[43]
	Hepatocellular carcinoma (HCC)	CircHIPK3 promotes HCC cell proliferation and migration via the circHIPK3/miR-124/AQP3 axis	┤miR-124, →AQP3	Up	[44]
	Diabetic retinopathy	CircHIPK3 promotes vascular endothelial cell proliferation and leads to vascular dysfunction	┤miR-30a-3p, →VEGFC, FZD4, WNT2	Up	[55]
	Bladder cancer	CircHIPK3 inhibits cell migration, invasion, and angiogenesis in bladder cancer	┤miR-558, →HPSE	Down	[45]
	Human oral squamous cell carcinoma (OSCC)	CircHIPK3 promotes the occurrence and development process of OSCC	┤miR-124	Up	[56]
	Non-small cell lung cancer	CircHIPK3 promotes cancer cell proliferation via the circHIPK3/miR-379/IGF1 pathway	┤miR-379, →IGF1	Up	[57]

"┤" – inhibitory roles; "→" – stimulatory roles. circRNA – circular RNA; c-Myc – Myc proto-oncogene, bHLH transcription factor c; Bcl-2 – B cell leukemia/lymphoma 2; ELF3 – ETS transcription factor 3; IGF2BP3 – insulin-like growth factor 2 mRNA-binding protein 3; ROCK1 – rho-associated protein kinase 1; CDK6 – rho-associated protein kinase; c-Myb – Myb proto-oncogene, transcription factor c; AQP3 – aquaporin 3; VEGFC – vascular endothelial growth factor C; FZD4 – frizzled class receptor 4; WNT2 – Wnt family member 2; HPSE – heparanase; IGF1 – insulin-like growth factor 1.

analysis revealed that hsa_circ_0002052 overexpression rescues the expression level suppressed by miR-1205 mimics [30]. In osteosarcoma tissues, the expression level of hsa_circ_0002052 and miR-1205, as well as of APC2 and miR-1205, both were inversely correlated, and overexpression of miR-1205 significantly abolished the effects of hsa_circ_0002052 overexpression on osteosarcoma cell proliferation, apoptosis, migration, and invasion, demonstrating that hsa_circ_0002052 suppresses osteosarcoma progression by inhibiting the miR-1205/APC2 axis of the Wnt/β-catenin pathway [30]. These data showed that hsa_circ_0002052 may act as a prognosis biomarker and therapeutic target of osteosarcoma.

Conclusions

Recent studies indicate that some circRNAs are deregulated in osteosarcoma tissues, which are closely associated with the

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clinical progress and prognosis of osteosarcoma patients [48]. Moreover, several osteosarcomatous that specifically express circRNAs were also reported to regulate the pathological process of other diseases, which provides new insights into the regulatory effect of these molecules in multiple diseases (Table 2). In the course of osteosarcoma progression, circRNAs can function as miRNA sponges and transcriptional regulators, modulating the expression of target miRNAs and proteins, thus regulating the proliferation, invasion, and metastasis of osteosarcoma cells. Because of their special annular structure, circRNAs have better stability and higher abundance, and these unique advantages make circRNAs promising diagnostic/prognostic biomarkers and novel therapeutic targets.

CircRNAs as biomarker for osteosarcoma

CircRNAs may be secreted and circulate in body fluids, and their annular structure gives them strong resistance to RNA exonucleases or RNase R. Recent studies suggested that circRNAs could serve as good candidates for tumor biomarkers with high specificity and high sensitivity [58,59]. In gastric cancer (GC), hsa_circ_0001649 is downregulated in tumor tissue, consequently leading to lower expression in serum of GC patients after surgery, being a potential recurrence biomarker [60]. Hsa_circ_0001445 was detected in plasma as a biomarker to distinguish HCC cases from healthy controls, as well as patients with cirrhosis or hepatitis B [61]. In metabolic osteopathy, hsa_circ_0001275 was found to serve as a potential diagnostic biomarker from peripheral blood mononuclear cells (PBMCs) of patients with postmenopausal osteoporosis [62]. Therefore, it would be worthwhile to explore the potential use of these circulatory circRNAs as biomarkers for osteosarcoma.

CircRNAs as therapeutic target for osteosarcoma

CircRNAs play an important role in the occurrence and progression of osteosarcoma, which determines their characteristics as potential therapeutic targets for treatment. CircRNAs can be targeted and silenced *in vitro* and *in vivo* and such approaches have long been evaluated for targeting critical cancer-associated genes in animal experiments. Although compared with coding RNAs, miRNAs, and lncRNAs, the studies on circRNAs are still in early stages, further development of targeting circRNAs for osteosarcoma treatment has great potential.

Conflicts of interest

None.

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