The role of non-coding RNAs in the regulation, diagnosis, prognosis and treatment of osteosarcoma (Review)

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Abstract. Osteosarcoma (OS) is the most common primary bone tumor worldwide. OS exhibits a range of aggressive behaviors, including early metastasis potential, rapid progression, poor clinical prognosis and insensitivity to chemoradiotherapy. Non-coding RNAs are transcripts that do not encode proteins. A significant number of studies published on OS have been focused on the aberrant expression of non-coding RNAs and their involvement in tumor initiation and progression. It has been confirmed that non-coding RNAs exert their regulatory functions at both the transcriptional and post-transcriptional level, which leads to tumor initiation or progression in OS. According to present knowledge, this review provides a state-of-the-art overview of the functions and mechanisms of microRNAs, long non-coding RNAs and circular RNAs in terms of their involvement with OS. The review also covers their potential clinical application in the diagnosis, prognosis and treatment of OS. It is hoped that the information presented in this review on the involvement of non-coding RNAs in OS will lead to a more comprehensive understanding of OS and provide a useful perspective on the potential diagnostic and therapeutic applications of non-coding RNAs for patients with OS.

Contents

1. Introduction

Osteosarcoma (OS) is considered to be the most commonly occurring type of primary bone tumor, with an estimated worldwide incidence of 3-4 new cases per million, which accounts for approximately 60% of the total number of cases of bone malignancy (1,2). OS is derived from the transformation of primitive mesenchymal cells, and typically occurs in the metaphyseal region of long bones, with a peak incidence among young individuals (3,4). OS exhibits a range of aggressive behaviors, including early metastasis potential, rapid progression, poor clinical prognosis and insensitivity to chemoradiotherapy, which collectively lead to a poor overall survival rate (5,6). Prior to the 1970s, surgical resection was the preferred treatment for OS, although, as the 5-year survival rate was <20%, it was insufficient as a means of therapy for numerous patients (7,8).

The current therapeutic strategy for OS includes neoadjuvant chemotherapy after surgical removal of the tumor and adjuvant chemotherapy with or without lesion metastasis,

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which has led to a marked increase in the survival rate to approximately 65% over the course of the last 30 years (9). However, despite notable improvements achieved in terms of surgical techniques and neoadjuvant chemotherapy, the overall survival time of patients with distant metastasis or multi-drug resistance cannot be effectively prolonged (10-12). Therefore, it is crucial to elucidate the underlying molecular mechanisms that are involved in the tumorigenesis and progression of OS, and to identify novel biomarkers for developing alternative therapies or improving the efficiency of existing treatments.

After having mapped out the transcriptional 'landscape' of the mammalian genome, findings showed that protein-coding mRNAs only account for 1.4% of the genome (13,14). Furthermore, by comparing the number of protein-coding genes with the genome size among different species, it was shown that the more complex eukaryotes carry a larger proportion of non-coding RNA (ncRNA) (15). These phenomena led to the suggestion that the ncRNAs in the human genome may contribute towards complex physiological and pathological processes. Based on a cutoff at 200 bases of length, ncRNAs are standardly categorized as short ncRNAs (sncRNAs) and long ncRNAs (lncRNAs) (16). MicroRNAs (miRNAs/miRs), as a typical class of sncRNAs, have been extensively studied. It has been confirmed that miRNAs exercise a regulatory role on the expression of protein-coding mRNAs, which leads to the initiation or progression of numerous diseases, including cancer (17,18). In addition, there are other types of ncRNAs, including small interfering RNAs (siRNAs), small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs). The lncRNAs, as observed from a wide spectrum of profiling in mammals, are a class of transcripts >200 nucleotides (nt) in length (13,19,20). Increasing evidence has demonstrated that lncRNAs fulfill functional roles in the occurrence and development of tumors (21,22). Along with the development of high-throughput sequencing technology and novel computational approaches, a set of circular RNAs (circRNAs) have recently been identified. It was revealed that circRNAs are involved in diverse pathological processes, including oncogenesis and tumor progression (23).

With the aim of encouraging further studies on these RNA species, the present review article provides a concise summary of ncRNAs and their biogenesis, their underlying molecular mechanisms and their potential clinical applications in OS. It is the authors' hope that associated research in the future may lead to a more comprehensive understanding of OS and present a reasonable perspective on the potential diagnostic and therapeutic application of ncRNAs in patients with OS.

2. miRNAs

Biogenesis and features of miRNAs. The first miRNA, lin-4, was discovered in *Caenorhabditis elegans* in 1993 and its involvement in various biological processes has been observed (24-26). Since then, thousands of miRNAs have been identified and studied over the course of the past 20 years. Further research has revealed that genes influenced by miRNAs exist in all metazoans and plants, suggesting that miRNA-associated regulation operates according to a highly conserved mechanism (27).

The biogenesis of miRNAs is a complex process, including nuclear synthesis and cytoplasmic synthesis with the involvement of a specific set of enzymes. First, in the nucleus, a primary miRNA (pri-miRNA) with special hairpin structures (AAAAA and 7MGpppG) is synthesized according to the transcriptional gene that encodes the miRNA by RNA polymerase II. Subsequently, the hairpin domain of the pri-miRNA is cleaved by an RNA-specific nuclease termed Drosha (ribonuclease III) to produce precursor miRNAs (pre-miRNAs) that possess a stem-ring structure and are 70-80 nt in length (28). Then, with the help of cytoplasmic transporter exportin-5, the pre-miRNAs are translocated from the nucleus to the cytoplasm. Within the cytoplasm, these pre-miRNAs are further cleaved into a miRNA duplex of ~19-23 nt by ribonuclease III (Dicerase) (29). The miRNA duplex consists of two strands: A mature miRNA strand and a passenger miRNA strand. After strand unwinding, the mature miRNA strand is transformed into a mature miRNA via its interaction with Argonaute protein, whereas the passenger miRNA is usually degraded (30).

Regulatory functions of miRNAs in OS. Aberrant expression of miRNAs has been reported as a common phenomenon occurring in a diversity of cancer types, including breast, lung, hepatocellular, colon and cervical cancer (31,32). miRNAs exert their regulatory role through interacting with their mRNA target genes. Normally, there are two mechanisms by which mature miRNAs are able to form RNA-induced silencing complex (RISC). In the first scenario, in cases where the miRNA is fully complementary to the target gene, the miRNA degrades the target gene. In the second scenario, where the miRNA is not fully complementary to target gene, miRNA combines with the 3'-untranslated region to inhibit translation of the target gene (33). In addition, it has been confirmed that one single miRNA may affect multiple mRNAs, or conversely, multiple miRNAs may affect one single mRNA (27). Through the mechanism described above, miRNAs are heavily involved in multiple instances of cancer occurrence and development, including proliferation, apoptosis and metastasis (34,35).

Similarly, certain miRNAs have been demonstrated to regulate malignancy by serving either as an oncogene or as an onco-suppressor in OS. miR-210-5p was recently shown to be upregulated in human OS tissues and cell lines, closely correlating with the advanced tumor-node-metastasis (TNM) stage, tumor size and pulmonary metastasis. Overexpressed miR-210-5p led to an increase in the rates of tumor invasion, migration and autophagy by suppressing the downstream target, phosphoinositide-3-kinase regulatory subunit 5 (PIK3R5). Of note, miR-210-5p-mediated autophagy facilitates miR-210-5p-induced tumor invasion and migration promotion via inhibiting the AKT/mTOR pathway (36). Another upregulated miRNA, miR-624-5p, has been identified in clinical OS specimens and cell lines. Further functional analysis suggested that miR-624-5p may promote cell proliferation, migration and invasion both *in vitro* and *in vivo*. Identified as the target gene of miR-624-5p, protein tyrosine phosphatase receptor type B (PTPRB) was found to be negatively correlated with miR-624-5p. Furthermore, PTPRB restored the effects of miR-624-5p on OS migration and invasion (37). miR-627-3p was shown to be downregulated in OS tissues

using biochip analysis, and was also shown to be expressed at a lower level in OS cell lines compared with human osteoblastic cells. Moreover, miR-627-3p significantly suppressed the expression and activity of pleiotrophin (PTN), and PTN affected the proliferation and migration of OS cells via regulation of a range of different proteins, including cyclin D1 and matrix metalloproteinase-2 (38). Also through microarray analysis, miR-15b was found to be markedly downregulated in the doxorubicin-resistance cell lines, KHOS and U-2OS (39). Furthermore, patients with OS who had high expression levels of miR-15b received a significantly improved clinical prognosis compared with those with low expression levels. Wee1, the direct target of miR-15b, was shown to mediate both the cytotoxic effect of doxorubicin and the multidrug-resistance capabilities in OS (39).

Although the participation of miRNAs in OS is a complex and multifactorial process, emerging evidence has strongly supported the involvement of numerous miRNAs in the processes of OS initiation and progression, including cell proliferation, apoptosis, immigration, invasion and drug resistance. These miRNAs and their roles are shown in Table I (36-51).

Role of miRNAs in the diagnosis, prognosis and treatment of OS. MiRNAs have consistently attracted a great deal of attention from researchers as putative diagnostic or prognostic biomarkers for OS due to their stability in the plasma and serum (52). miR-21 overexpression has been shown to be strongly associated with advanced Enneking stage, chemotherapeutic resistance and an unfavorable prognosis, and therefore miR-21 may be used as an individual marker for OS staging and prognosis (53). Low levels of miR-101 have been observed in serum samples from patients with OS; however, the miR-101 expression levels reverted to significantly higher levels following treatment (54). miR-195-5p and miR-199a-3p have been shown to have remarkable potential in terms of distinguishing between metastatic and non-metastatic statuses in patients with OS, whereas miR-320a and miR-199a-3p were associated with the histological subtype (55).

At present, the standard clinical treatments mainly comprise surgical resection and neoadjuvant therapy. The involvement of miRNAs in the initiation and progression of OS renders miRNAs suitable as possible therapeutic targets. The corresponding approach would involve the use of miRNA mimics to substitute for the loss of expression of a tumor-suppressor miRNA or to block the expression of an oncomiR using oligonucleotides or anti-viral constructs (56). A mimic of miR-34 as a tumor suppressor for cancer treatment was entered into Phase I clinical trials (57). Furthermore, it was identified that a miR-34 mimic significantly suppressed lung metastasis in OS mouse models, strongly suggesting that miR-34 may serve as a potential therapeutic target (58).

3. lncRNAs

Biogenesis and features of lncRNA. lncRNAs form a large subgroup of ncRNAs with transcripts >200 bases in length that lack the ability to encode proteins. For the most part, they are transcribed by RNA polymerase II, the same as mRNAs, and they are often 5'-capped, polyadenylated and spliced without a translated open reading frame (59,60). Based on their location with the neighboring protein-coding genes, they can be divided into the following five classes: Sense, antisense, bidirectional, intronic and intergenic lncRNAs (61-63). lncRNAs are also characterized by their low abundance and through the tissue- and developmental stage-specific manner of their expression (64,65).

Regulatory functions of lncRNAs in OS. Similar to miRNAs, numerous lncRNAs have been demonstrated to have key roles as contributors to tumor initiation or progression. The manner in which a given lncRNA exerts its regulatory effect depends on its subcellular localization. lncRNAs in the cytoplasm that share miRNA response elements with mRNAs contain similar sequences to these target-coding RNAs and inhibit the interactions between miRNAs and mRNAs. These lncRNAs, which are termed competing endogenous RNAs (ceRNAs), act as 'sponges' for miRNAs and regulate the process of translation mediated by miRNAs on their target mRNAs (66). lncRNAs in the nucleus mainly act at the epigenetic and genetic levels by binding to the transcription preinitiation complex at the promoter (67).

The lncRNA DANCR has been shown to be elevated in OS tissue specimens and cell lines and is closely correlated with poor prognosis among clinical patients. DANCR serves as an oncogene, regulating ROCK1-mediated proliferation and metastasis through sequestering both miR-335-5p and miR-1972 as a ceRNA (68). The lncRNA HIF1A-AS2 has also been shown to be upregulated in OS, and is associated with poor survival. HIF1A-AS2 regulates the tumorigenesis of OS, as demonstrated by its effects on cell proliferation, cell cycle progression and invasion, through 'sponging' miR-129-5p (69). In OS, the lncRNA TTN-AS1 has been shown to facilitate cell growth, apoptosis and drug resistance via the miR-134-5p/MBTD1 axis (70). By contrast, the lncRNA TTN-AS1 also acts as a ceRNA on miRNA-376a, enhancing the malignancy of OS via upregulating dickkopf-1 (71). Other confirmed lncRNAs are presented in Table II (68-79).

Role of lncRNAs in the diagnosis, prognosis and treatment of OS. At present, the main surveillance methods of OS are limited to physical examination, blood biochemistry and radiographic examination (80). Due to the lack of an effective and noninvasive measure to monitor patient status or predict overall survival, lncRNAs are considered as potential candidates for prognosis prediction and treatment guidance. The level of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) has been shown to be upregulated in 162 OS tissues, closely correlating with advanced clinical stage, distant metastasis and shorter survival times. Therefore, MALAT1 is able to serve as an independent prognostic factor for OS (81). The clinical potential of taurine-upregulated gene 1 (*TUG1*) has also been demonstrated through its marked elevation in patients with OS progression and relapse. Furthermore, the serum levels of TUG1 were shown to be decreased after surgical resection of OS tissues in postoperative patients (82). The most common obstacles in the treatment of OS are the poor therapeutic response to traditional chemo- and radio-therapies and the emergence of resistance during treatment (83). lncRNAs that mediate acquired resistance are potential candidates as targets for OS therapies.

Table I. Examples of the involvement of miRNAs in OS.

Name	Expression	Role	Related molecules	Phenotypes affected	Experimental models	(Refs.)
miR-627-3p	↓	Tumor suppressor	PTN	Proliferation (-), migration (-), invasion (-)	HOS, U2OS, MG63 and MG63/dox cells	(38)
miR-15b	↓	Tumor suppressor	Wee1	Drug resistance (+)	KHOS, U-2OS and corresponding drug-resistant cells	(39)
miR-382	↓	Tumor suppressor	KLF12 and HIPK3	Cell growth (-), chemosensitivity (+)	MNNG/HOS, U2OS and MG63 cells	(40)
miR-145	↓	Tumor suppressor	FLI-1	Cell growth (-)	HOS, Saos-2, U2OS and MG-63	(41)
miR-143	↓	Tumor suppressor	MMP-13	Metastasis (-)	143B cells	(42)
miR-26a	↓	Tumor suppressor	Jagged1	Cell growth (-)	U2OS, MG63, Saos-2 and 143B cells	(43)
miR-29b	↓	Tumor suppressor	CDK6	Proliferation (-), migration (-)	MG-63 cells	(44)
miR-539	↓	Tumor suppressor	MMP-8	Migration (-), invasion (-)	MG-63, U2OS and Saos2 cells	(45)
miR-210-5p	↑	Oncogene	PIK3R5	Migration (+), invasion (+), autophagy (+)	HOS, Saos-2, SW1353, U2OS, and MG63 cells	(36)
miR-624-5p	↑	Oncogene	PTPRB	Migration (+), invasion (+)	U2OS, MG63, SW1353, HOS, and Saos-2 cells	(37)
miR-21	↑	Oncogene	Caspase-8	Apoptosis (-)	Saos-2 cells	(46)
miR-543	↑	Oncogene	PRMT9	Proliferation (+)	Saos2, MNNG/HOS, U2OS and MG63 cells	(47)
miR-27a	↑	Oncogene	CBFA2T3	Metastasis (+)	HOS, KHOS, SAOS2, 143B and U2OS cells	(48)
miR-191	↑	Oncogene	CHEK2	Proliferation (+)	Saos2 and MG-63 cells	(49)
miR-199a-5p	↑	Oncogene	PIAS3 and p27	Cell growth (+)	MG63, Saos-2 and 143B cells	(50)
miR-9	↑	Oncogene	GCIP	Cell growth (+)	U2OS and HOS cells	(51)

↓, downregulated; ↑, upregulated; PTN, pleiotrophin; Wee1, WEE1 G2 checkpoint kinase; KLF12, Kruppel-like factor 12; HIPK3, homeodomain interacting protein kinase 3; FLI-1, friend leukemia virus integration 1; MMP-13, matrix metallopeptidase 13; CDK6, cyclin-dependent kinase 6; MMP-8, matrix metallopeptidase 8; PIK3R5, phosphoinositide-3-kinase regulatory subunit 5; PRMT9, protein arginine methyltransferase 9; CBFA2T3, CBFA2/RUNX1 partner transcriptional co-repressor 3; CHEK2, checkpoint kinase 2; PIAS3, protein inhibitor of activated STAT 3; GCIP, Grap2 and cyclin D interacting protein.

Table II. Examples of the involvement of lncRNAs in OS.

Name	Expression	Role	Related molecules	Phenotypes affected	Experimental models	(Refs.)
CASC2	↓	Tumor suppressor	miR-181a and RASSF6	Proliferation (-), invasion (-)	MG-63, SW1353, Saos-2, SOSP-9607 and U2OS cells	(72)
CTA	↓	Tumor suppressor	miR-210	Drug resistance (+)	Saos-2, U-2OS and MG-63 cells	(73)
GAS5	↓	Tumor suppressor	Downregulate ARHI via miR-221	Cell growth (-), migration (-), EMT (-)	KHOS, 143B, SAOS-2, U2OS, and MG-63 cells	(74)
FENDRR	↓	Tumor suppressor	ABCB1 and ABCC1	Apoptosis (+), drug resistance (-)	MG63, SaoS2 and HOS cells	(75)
DANCR	↑	Oncogene	Upregulate ROCK1 via miR-335-5p and miR-1972	Proliferation (+), invasion (+), migration (+)	MG-63, U2OS, MNNG/HOS and 143B cells	(68)
HIF1A-AS2	↑	Oncogene	miR-129-5p	Proliferation (+), invasion (+)	U2OS, SoSP-M, SaOS2, MG-63 cells	(69)
TTN-AS1	↑	Oncogene	miR-134-5p/MBTD1 axis and miR-376a/DKK1 axis	Proliferation (+), apoptosis (+), invasion (+), migration (+) drug resistance (-)	Saos-2, HOS, MG-63 and U2OS cells	(70,71)
H19	↑	Oncogene	Inactivate NF-κB pathway via PI3K/AKT signaling pathway	Migration (+), invasion (+)	MG-63, U2OS and SAOS-2 cells	(76)
SNHG12	↑	Oncogene	Upregulate Notch2 via miR-195-5p	Proliferation (+), invasion (+), migration (+)	143B, U2OS, MG63 and HOS cells	(77)
NEAT1	↑	Oncogene	Upregulate HOXA13 via miR-34a-5p	Proliferation (+), cells apoptosis (-)	Saos2, MG63, U2OS, SJSA1, and HOS cells	(78)
APTR	↑	Oncogene	Upregulate YAP1 via miR-132-3p	Proliferation (+), invasion (+), migration (+), apoptosis (-)	MG63 and 143B	(79)

↓, downregulated; ↑, upregulated; CASC2, cancer susceptibility 2; RASSF6, Ras association domain family member 6; GAS5, growth arrest specific 5; ARHI, aplasia Ras homolog member 1; FENDRR, FOXF1 adjacent non-coding developmental regulatory RNA; ABCB1, ATP binding cassette subfamily B member 1; ABCC1, ATP binding cassette subfamily C member 1; DANCR, differentiation antagonizing non-protein coding RNA; ROCK1, Rho associated coiled-coil containing protein kinase 1; HIF1A-AS2, hypoxia inducible factor 1 subunit α antisense RNA 2; TTN-AS1, titin antisense RNA 1; MBTD1, mbt domain containing 1; DKK1, dickkopf WNT signaling pathway inhibitor 1; PI3K, plasmodium gallinaceum phosphatidylinositol 3-kinase; SNHG12, small nucleolar RNA host gene 12; NOTCH2, notch receptor 2; NEAT1, nuclear paraspeckle assembly transcript 1; HOXA13, homeobox A13; APTR, Alu-mediated CDKN1A/p21 transcriptional regulator; YAP1, Yes1-associated transcriptional regulator.

4. circRNAs

Biogenesis and features of circRNAs. CircRNAs, a novel class of non-protein-coding RNAs, were first discovered in the 1970s, and were then considered as 'junk' molecules with little functional potential (84). They are generated from pre-mRNAs through back-splicing, and are expressed in a tissue- and developmental stage-specific manner (85,86). In addition, they are evolutionarily conserved and highly abundant in the brain. Unlike traditional linear RNAs, circRNAs are characterized by a continuous covalently closed loop structure lacking either a 5'-cap or a 3'-polyadenylated tail, which gives them stronger resistance to ribonucleases compared with their corresponding linear counterparts (87,88).

Regulatory functions of circRNAs in OS. Emerging evidence has shown that circRNAs fulfill essential roles in both physiological and pathological processes, including oncogenesis and tumor progression (23). circRNAs have multiple functions, such as regulating gene expression at the transcriptional or post-transcriptional level by interacting with miRNAs as 'sponges', binding to RNA-binding protein and initiating protein translation in a splicing-dependent, cap-independent manner (89,90). The circRNA circTADA2A has been reported to be highly upregulated in OS, and acts as a sponge for the miRNA miR-203a-3p, which regulates CREB3 expression to promote the proliferation, migration and invasion of OS cells *in vitro* (91). The circRNA hsa_circ_0001564, detected through circRNA microarray analysis, has been shown to be upregulated in OS tissues. A further study revealed that hsa_circ_001564 aggravates OS proliferation and apoptosis via sponging miR-29c-3p (92). circPVT1 facilitates the doxorubicin and cisplatin resistance of OS via increasing the expression of the classical drug resistance-associated gene, *ABCB1* (93). In patients with OS, circNASP expression was found to be positively correlated with tumor size and lung metastasis. Upregulated circNASP acts as a sponge of miR-1253, targeting the transcription factor FOXF1 to markedly promote the proliferation, cell cycle progression and invasion of OS cells (94). Other novel circRNAs are listed in Table III (91-103).

Role of circRNAs in the diagnosis, prognosis and treatment of OS. CircRNAs can be secreted in body fluids where they are circulated, and the structures of circRNAs are geared towards a high level of resistance to cleavage by RNA exonucleases or ribonuclease R. These features, along with high specificity and sensitivity, demonstrate that circRNAs may serve as good candidates for OS (104,105). For example, the high expression level of serum circPVT1 enabled patients with OS to be distinguished from healthy individuals, suggesting that circPVT1 may be more reliable as a diagnostic biomarker compared to the traditional biomarker alkaline phosphatase (93). In another study, evaluated expression levels of circUBAP2 were found via Kaplan-Meier survival analysis to be correlated with reduced survival and poor prognosis, and they were also significantly correlated with the tumor stages (106).

CircRNAs, as ceRNAs, are natural miRNA inhibitors that bind to their corresponding miRNA to regulate the malignant behavior of OS. This property ensures that circRNAs have great potential in terms of therapeutic strategies. Recently, a newly designed artificial miRNA sponge has been developed. This artificial circRNA can sponge multiple miR-21 molecules, and has been reported to upregulate the expression of the tumor suppressor gene *DAXX* to inhibit the proliferation of gastric cancer cells (107).

5. Conclusions and perspectives

The identification of ncRNAs and their role in cancer initiation and progression has provided revolutionary insights into how the research efforts for OS may be directed. MiRNAs exert their regulatory functions via RISC, whereas lncRNAs and circRNAs function according to mechanisms involving ceRNAs (Fig. 1). However, since the majority of these studies have focused on miRNAs, lncRNAs and circRNAs, rather than other types of ncRNA (such as snoRNAs), the data remain incomplete. Of the numerous human ncRNAs, only a few have been thoroughly studied, and only a limited number of these have an important biological impact. The RNA world is much more complicated than was once considered to be the case, and the clinical environment for OS progression should be greatly improved if more research is devoted to the study of ncRNAs and their involvement in this type of cancer.

Although the significant clinical potential of ncRNAs as biomarkers in OS has been acknowledged, there exist several limitations. In most of the studies that have been performed to date, the cohort of patients with OS was relatively small; thus, long-term, controlled and large-sample experiments are required. In addition, the detected ncRNA biomarkers do not perform entirely consistently even for a particular cancer type, which is an important obstacle in terms of their usefulness as biomarkers. Therefore, it is necessary to identify combinations of several ncRNAs with a high degree of specificity and sensitivity, and to develop a standardized approach in methodology to normalize the expression of ncRNAs.

NcRNAs possess a unique advantage in terms of their clinical application in OS treatment. A single ncRNA simultaneously targets multiple downstream factors and is involved in multiple signaling pathways, which brings important benefits for refractory cancers with genomic heterogeneity. However, there are certain disadvantages or challenges associated with ncRNA-targeted treatment strategy. First, they may break the balance of gene expression profiles in cells due to unrelated genes being targeted by the same ncRNAs. It has been observed that miRNAs may exert different effects, which means that miRNA antagonists or mimics must be carefully selected according to different conditions. Furthermore, the existence of off-target effects for ncRNA antagonists cannot be ignored. Therefore, extensive toxicity studies and preclinical safety requirements should be assessed before an ncRNA-based therapeutic approach may be considered as being appropriate for patients with OS (108). At present, researchers have also made efforts to develop effective drug delivery systems for chemotherapy. The reduction-responsive polypeptide micelles were developed as multifunctional nanoparticle-based drug delivery systems based on methoxy poly-block-poly copolymers. These micelles can selectively accumulate in OS tumors, which induces antitumor effects with less systematic toxicity (109). As

Table III. The expression and function of circRNAs in OS.

Name	Expression	Role	Related molecules	Phenotypes affected	Experimental models	(Refs.)
circ_0002052	↓	Tumor suppressor	Inactivate Wnt/β-catenin pathway via miR-1205/APC2 axis	Proliferation (-), apoptosis (+), migration (-), invasion (-)	HOS, U2OS, MG63 and 143B cells	(95)
circITCH	↓	Tumor suppressor	miR-22 and miR-7	Proliferation (-), apoptosis (+), migration (-), invasion (-)	MG63, U2OS, Saos-2 and SJSA-1 cells	(96,97)
circ_HIPK3	↓	Tumor suppressor	-	Proliferation (-), migration (-), invasion (-)	SaoS2, HOS, KH-OS, MG63, 143B and U2OS cells	(98)
circTADA2A	↑	Oncogene	miR-203a-3p/CREB3 axis	Proliferation (+), migration (+), invasion (+)	HOS, 143B, MG-63, U2OS and SJSA-1 cells	(91)
circ_0001564	↑	Oncogene	miR-29c-3p	Proliferation (+), apoptosis (-)	U2OS, Saos-2, HOS and MG-63 cells	(92)
circPVT1	↑	Oncogene	ABCB1	Drug resistance (+)	SaoS2, KHOS, U2OS, MG63 cells	(93)
circNASP	↑	Oncogene	miR-1253/FOXF1 axis	Proliferation (+), invasion (+)	143B and MG63 cells	(94)
circ_0102049	↑	Oncogene	upregulate MDM2 via miR-1304-5p	Cell growth (+), migration (-), invasion (+), apoptosis (-)	MG63, HOS, Saos2, and U2OS cells	(99)
circ_0001721	↑	Oncogene	miR-569 and miR-599	Proliferation (+), apoptosis (-), migration (+), invasion (+)	HOS, Saos2, MG63 and U2OS cells	(100)
circGLI2	↑	Oncogene	miR125b-5p	Proliferation (+), migration (+), invasion (+)	MG63, Saos-2, HOS and U2OS cells	(101)
circ_0001785	↑	Oncogene	regulate HOXB2 via miR-1200	Proliferation (+), apoptosis (-)	Saos2, U2OS, SJSA1, HOS and MG63 cells	(102)
circ_0060428	↑	Oncogene	regulate RBPJ via miR-375	Proliferation (+), apoptosis (-)	U2OS, 143B, SAOS-2 and HOS cells	(103)

↓, downregulated; ↑, upregulated; ITCH, itchy E3 ubiquitin protein ligase; HIPK3, homeodomain interacting protein kinase 3; TADA2A, transcriptional adaptor 2A; CREB3, cAMP responsive element binding protein 3; NASP, nuclear autoantigenic sperm protein; FOXF1, forkhead box F1; GLI2, GLI family zinc finger 2; HOXB2, homeobox B2; RBPJ, recombination signal binding protein for immunoglobulin κ J region.

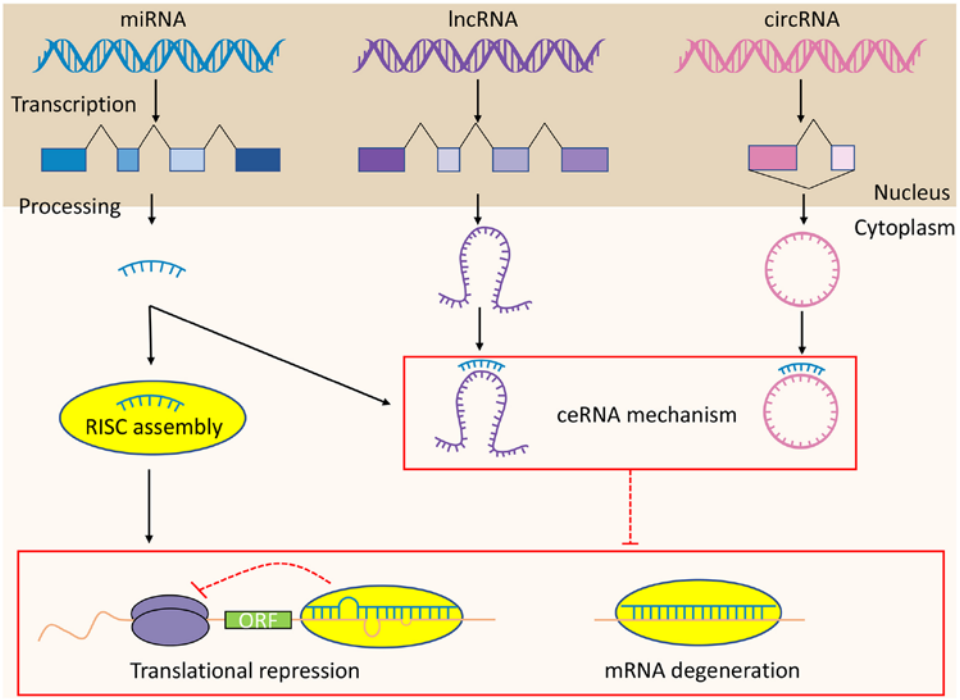

Figure 1. Regulation processes of miRNAs, lncRNAs and circRNAs. The mature miRNA is attached to the RISC and binds with partial complementarity to the 3'-UTR of target mRNAs to mediate translational repression. Alternatively, mature miRNAs may bind with perfect complementarity to the 3'-UTR of target mRNAs, which undergo cleavage, consequently leading to their degradation. ceRNAs, similar to lncRNAs and circRNAs, are able to combine with miRNAs through MREs to affect gene regulation mediated by miRNAs. miRNAs, microRNAs; lncRNA, long non-coding RNA; circRNA, circular RNA; UTR, untranslated region; RISC, RNA-induced silencing complex; ceRNA, competing endogenous RNA; MREs, miRNA-response elements.

for nucleic acid therapeutics, approaches of delivery systems for targeting ncRNAs have been developing at a rapid pace. There are some general problems of nucleic acid delivery strategies including short half-life, off-target effects and low transfection efficiency in RNA delivery, which makes nucleic acid drugs remain at a low bioavailability *in vivo* (110,111). To overcome the aforementioned obstacles, a variety of ncRNA carriers or systems have been investigated, including nanoparticles, ncRNA modification, and oncolytic adenovirus strategy (112). Several of the delivery strategies have been applied in the research of hepatic carcinoma (113,114). Despite the lack of nucleic acid drug delivery strategies and associated research in OS, nucleic acid therapy may prove beneficial for OS treatment through further study.

In the present review, we have made detailed predictions on the future research directions:

i) One single ncRNA is able to affect multiple downstream target molecules associated with cancer development, and one single downstream target can be regulated by multiple upstream molecules. For instance, the lncRNA DANCR promotes cell proliferation and metastasis via sponging miR-335-5p and miR-1972 in OS (68). Therefore, understanding the complicated connections between the ncRNA regulatory networks, as well as determining some important core ncRNAs in OS, requires further investigation.

ii) Typically, researchers obtain tissue samples for early diagnosis and prognosis via surgery resections, which is a difficult and inconvenient procedure. Findings have shown that the serum expression of the lncRNA UCA1 was significantly higher in patients with OS compared with healthy controls. In addition, the upregulation of UCA1 was correlated with clinical stage and metastasis (115). Although the data reported are only preliminary, it is possible to predict that liquid biopsy, such as human peripheral blood, is a promising non-invasive technique for OS diagnosis and prognosis in clinical practice. Furthermore, clinical specimens can vary from puncture fluid to sputum if patients have lung metastasis.

iii) At present, a limited number of proteins or peptides encoded by ncRNAs have been verified, and these have important biological and/or pathological functions in the occurrence and development of different tumors. A conserved 53-amino-acid peptide encoded by the lncRNA HOXB-AS3 was shown to suppress the proliferation, migration, invasion of colon cancer cells and tumor growth both *in vitro* and *in vivo* (116). FBXW7-185aa is encoded by the circRNA FBXW7, and inhibits glioma growth (117). Therefore, it is important to distinguish whether ncRNAs exert functions by acting directly as RNA molecules, or through encoding peptides or proteins.

iv) Avoiding immune surveillance is an important hallmark of tumor initiation and progression (118). Recent findings have shown that ncRNAs are able to facilitate tumor immune escape to enhance malignant behaviors. The PD-1/PD-L1 pathway provides a key immunosuppressive mechanism for cancer cells, and miR-140 is associated with anti-tumor immunity via its effects on the PD-L1/PD-1 immune checkpoint signaling pathway in OS (119). At present, however, there are only limited numbers of studies on ncRNAs and their association with the tumor immune response in OS. Thus, further investigation to

develop promising immunotherapies for patients with OS is necessary.

v) From a technical perspective, the research technology of circular RNA (circRNA) is similar to the classic RNA research technology, such as RT-PCR, qPCR, FISH (RNA positioning) and NB (northern blot) (RNA expression). However, with the emergence of new technologies, the research methods of circular RNA (circRNA) have become more abundant, such as using CRISPR/Cas13 system to efficiently knock down the expression of circular RNA without affecting the expression of its parental linear mRNA (120).

In conclusion, the investigation of emerging functional ncRNAs has led to a deeper understanding of the pathologies that control initiation and progression in OS. Moreover, the potential applications of ncRNAs in the diagnosis, prognosis and therapy of OS have been revealed in recent years. Further efforts, however, are required to elucidate the ncRNA-associated regulatory mechanisms and to establish ncRNA-targeted therapeutic options.

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Authors' contributions

GY, YW, HS and HL made substantial contributions to the concept and design of the review, collected information and wrote the manuscript. RW and WH collected references, and reviewed and edited the manuscript. HS was the major contributor in drafting and revising the manuscript. The authenticity of all the raw data have been assessed by GY and YW to ensure its legitimacy. All authors read and approved the final version of this manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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