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Advances in the Biological Functions and Mechanisms of miRNAs in the Development of Osteosarcoma

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Abstract

Osteosarcoma is one of the most common primary malignant bone tumors, mainly occurring in children and adolescents, and is characterized by high morbidity and poor prognosis. MicroRNAs, a class of noncoding RNAs consisting of 19 to 25 nucleotides, are involved in cell proliferation, invasion, metastasis, and apoptosis to regulate the development and progression of osteosarcoma. Studies have found that microRNAs are closely related to the diagnosis, treatment, and prognosis of osteosarcoma patients and have an important role in improving drug resistance in osteosarcoma. This paper reviews the role of microRNAs in the pathogenesis of osteosarcoma and their clinical value, aiming to provide a new research direction for diagnosing and treating osteosarcoma and achieving a better prognosis.

Keywords

microRNAs, osteosarcoma, biomarkers, targeted therapy, molecular mechanisms

Abbreviations

CDK2, cyclin-dependent kinase 2; CDK4, cyclin-dependent kinase 4; CDKN1A, cyclin-dependent kinase inhibitor 1A; CDKN1B, cyclin-dependent kinase inhibitor 1B; DKK1, Dickkopf WNT Signaling Pathway Inhibitor 1; DKK3, Dickkopf WNT Signaling Pathway Inhibitor 3; DNMT3B, DNA Methyltransferase 3 Beta; E2F5, E2F Transcription Factor 5; EBF2, Early B-Cell Factor-2; FBXW11, F-box and WD repeat domain containing 11; FBXW7, F-Box And WD Repeat Domain Containing 7; GPX4, glutathione peroxidase 4; HES1, Hes Family BHLH Transcription Factor 1; HIF-1α, Hypoxia inducible factor-1α; IFI44L, interferon-induced protein 44-like; IRF1, interferon regulatory factor 1; IRF2, interferon regulatory factor 2; ITGAV, integrin subunit alpha V; JMJD2C, Jumonji C domain-containing oxygenase D2C; KLF12, Kruppel Like Factor 12; MAFG, MAF BZIP Transcription Factor G; MAKPL, NF-κB-activating protein-like; PTEN, phosphatase and tensin homolog; PTPN14, Non-receptor tyrosine phosphatase 14; ROCK1, Rho-associated protein kinase 1; Runx2, Runt-related transcription factor 2; SIX1, sine oculis homeobox 1; SOX4, SRY-Box Transcription Factor 4; STAT3, Signal transducer and activator of transcription 3; TAZ, Transcriptional co-activator with PDZ-binding motif; TGFB1, transforming growth factor-β1; TRAF3, TNF receptor-associated factor 3; VEPH1, Ventricular Zone Expressed PH Domain Containing 1; ZIF, zeolitic imidazolate framework

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Introduction

Osteosarcoma (OS) originates from primitive mesenchymal cells, and it is the most common malignant bone tumor in children and adolescents.[1] The most common sites of osteosarcoma are the metaphysis of long bones, especially the distal femur, proximal tibia, and proximal humerus.[2,3] Most patients with OS have pain and swelling in the affected area.[4] Due to surgical resection, combined chemotherapy and targeted radiotherapy in

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treating osteosarcoma, the 5-year survival rate for patients without metastases can be around 60% to 70%.[5,6] However, metastases and recurrences often lead to a poor prognosis, so the 5-year survival rate for OS patients with metastases or recurrences is only 10% to 20%.[7,8] The malignant grade of osteosarcoma is high, the micro-lesion metastasis may be possible in the diagnosis, and lung tissue is a common metastatic site.[9] At the same time, osteosarcoma cells can become resistant to various chemotherapeutic agents during treatment, which poses a huge dilemma for the clinical management of osteosarcoma.[10] Although overall survival is universally recognized as the gold standard when assessing prognostic information or measuring treatment effects in clinical research, the complexity of cancer death, including invasion, recurrence, and metastasis, still limits the practicality and reliability of OS in the estimation of cancer progress and prognosis.[11] In addition, a novel technique, positron emission tomography/computed tomography (PET/CT), which is widely used in clinical practice, shows more accuracy, sensitivity, and specificity in the diagnosis of osteosarcoma. However, finance and cost limits have prevented its widespread use.[12,13] Therefore, it is necessary to conduct in-depth research on osteosarcoma diagnosis and treatment options and actively pursue clinical translation to improve the current diagnosis and treatment methods and obtain a better prognosis.

MicroRNAs (miRNAs) are a class of highly conserved endogenous non-coding RNAs, approximately 19 to 25 nucleotides in length.[14] They perform their biological functions mainly through the regulation of gene expression.[15] Abnormal expression of miRNAs is associated with various types of cancer, and these abnormal miRNAs often function as oncogenes or tumor suppressors during cancer development and progression.[16–18] Therefore, attempts can be made to improve the expression of aberrant miRNAs by inhibiting the function of highly expressed miRNAs in cancer or by supplementing the amount of downstream specific products of lowly expressed miRNAs through various pathways, thereby inhibiting the development and progression of cancer.[19,20] Recently, many studies have shown that numerous miRNAs are either overexpressed or underexpressed in osteosarcoma and are often associated with the entire process of tumor development.[21] Therefore, this paper reviews the research progress of miRNAs in osteosarcoma to explore the potential application of miRNAs in the diagnosis and treatment of osteosarcoma and prognosis and lay the theoretical foundation for the update of clinical diagnosis and treatment methods.

The Production and Biological Functions of miRNAs

The Generation of miRNAs

Most miRNAs are produced through the typical miRNAs biogenesis pathway.[22] MicroRNA genes are usually expressed as single transcription units, and mature miRNAs are produced through 2 steps, nuclear and cytoplasmic synthesis, and this process requires the involvement of multiple enzymes.[23] The formation of miRNAs begins in the nucleus, where most miRNAs are transcribed by RNA polymerase II (Pol II), producing primary miRNAs (pri-miRNAs) that encode miRNAs sequences in a "hairpin" structure.[24] Pri-miRNAs are excised in the nucleus by the Drosha-DGCR8 complex to produce precursor miRNAs (pre-miRNAs) that retain their "hairpin" structure.[23,25] The pre-miRNAs are then translocated to the top of the hairpin to produce precursor miRNAs (pre-miRNAs) that still retain their "hairpin" structure. The pre-miRNAs are then transported to the cytoplasm by the transporter protein Exportin-5. In the cytoplasm, pre-miRNAs bind to the double-stranded RNA (dsRNA) endoribonuclease/transactivation response (TAR) RNA binding protein (DICER/TRBP) complex, which cleaves the hairpin and produces a complementary RNA duplex. One of the nucleotide chains preferentially binds to the RNA-induced silencing complex (RISC) containing the Argonaut (Ago) protein to form the miRNA-RISC complex, while the other chain is degraded. The choice of the strand is largely dependent on the local thermodynamic stability of the miRNAs duplex—RISC tends to load onto the less stably paired 5′ end. This thermodynamic difference occurs partly because miRNAs tend to start with uracil and partly because miRNAs duplexes contain mismatches and bumps that favor the loading of miRNAs strands into RISC. Finally, Argonaut proteins guide the miRNAs strand to the 3′-untranslated region (3′-UTR) of the target sequence on the mRNAs and allow it to bind to the RISC.[26–29] In the middle, the structural characteristics of the 5′-UTR in mRNA, including the upstream UTR, secondary structure, start codon, open-reading frame (ORF), and ribosome binding sites, regulate the efficiency of translation.[30] Although most transcription regulation of mRNA takes place in 5′-UTRs, the 3′-UTRs of mRNAs play an important role in the posttranscriptional regulation of gene expression through the presence of cis-acting elements and through interaction with miRNAs.[30,31] The commonly recognized function of miRNAs is through interacting with the 3′-UTR regions of targeted mRNAs and hinders gene expression, which subsequently leads to the alteration of multiple cellular functions, including growth, migration, and angiogenesis.[32] Moreover, the 3′-UTR of mRNA contains terminal processing signals, and its miRNA targeted binding region and AU-rich region regulate gene expression at the translational level.[33] In addition, it has been widely reported that miRNA expression is remarkably deregulated in cancer.[34] So any mutations in miRNAs has the power to attack specific domains such as the coding region of mRNA domains that serve as binding targets and result in oncogenic or tumor suppressor activity of the miRNA.[34] miRNAs can also be secreted outside the cell and may be involved in intercellular communication.[35] Extracellular miRNAs are stably present within vesicles, including apoptotic vesicles and exosomes, or bound to RNA-binding proteins such as AGO and HDL.[36] The biogenesis pathway of miRNAs is depicted in Figure 1.

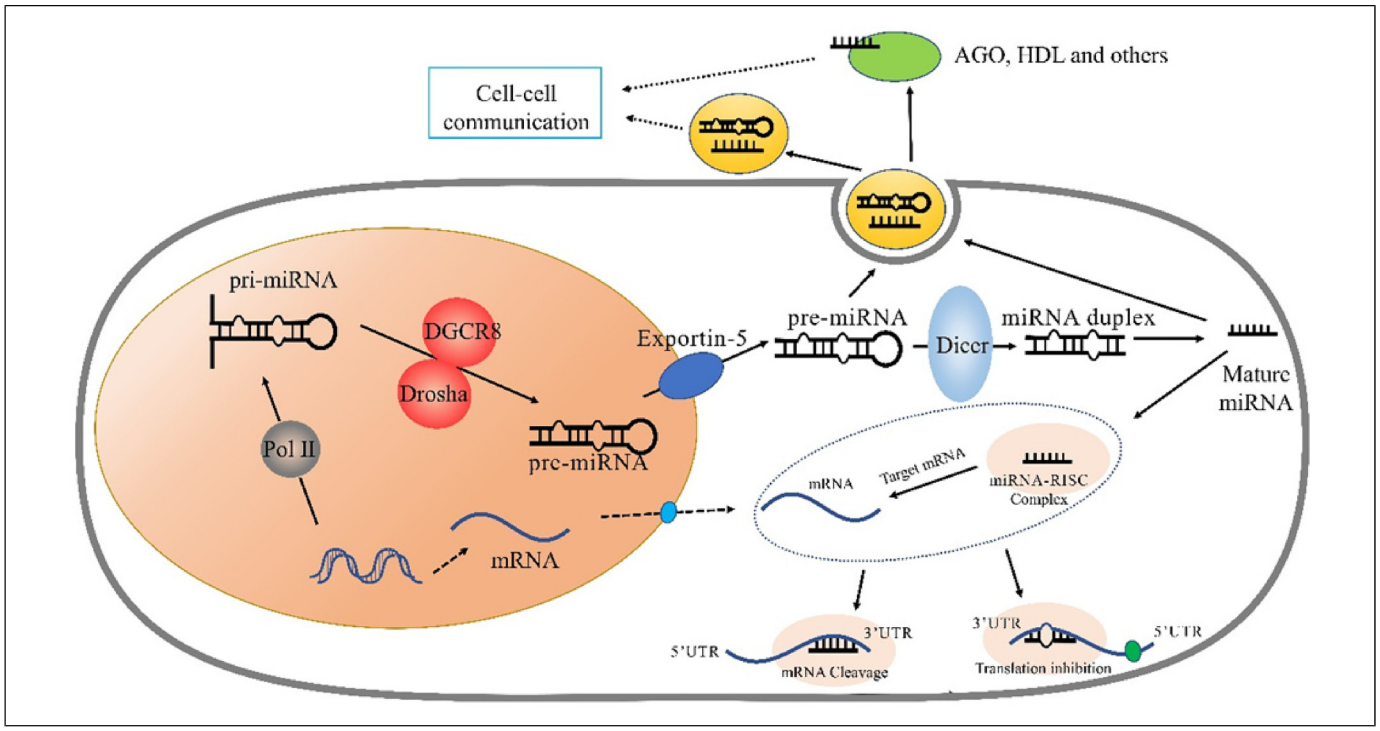

Figure 1. The biogenesis of miRNA begins in the nucleus. First of all, the miRNA gene is transcribed by pol II to produce pri-miRNA. Subsequently, a complex of Drosha and DGCR8 proteins cleave pri-miRNA to produce pre-miRNA. Then, pre-miRNA is transported to the cytoplasm by Exportin-5, and Dicer guides TRBP and PACT to further process pre-miRNA to produce miRNA duplex. Finally, the guide chain of miRNA duplex and AGO protein is loaded into RISC to recognize the target mRNA through sequence complementarity, and gene silencing is caused by mRNA degradation or translation inhibition. In addition, miRNA is also secreted out of the cell and may be involved in intercellular communication.

The Biological Functions of miRNAs

Among the various types of small molecule RNAs, miRNAs have a wide range of gene regulatory functions.[37] Although miRNA genes only account for 1% to 3% of the human genome.[38] However, it is estimated that miRNAs regulate about 30% to 60% of protein-coding genes in the human body.[39,40] miRNAs can be detected in cells and various biological fluids, such as plasma, serum, and follicular fluid.[41–43] Mature miRNAs can form complexes that induce RNA to silence through 2 processes: (1) when miRNAs are not perfectly complementarily paired with sequences in the 3′-UTR of target mRNAs, miRNAs inhibit protein synthesis by repressing translation or promoting the deadenylation and decay of mRNAs; (2) when miRNAs are almost perfectly complementarily paired with target mRNAs, these mRNAs inhibit protein synthesis by repressing translation or promoting the decay of mRNAs. When paired, these mRNAs are cleaved and degraded by nucleic acid endonucleases.[44] MiRNAs can regulate normal cellular genetic activities such as differentiation, proliferation, apoptosis, intracellular signaling, gene expression, tissue development, material metabolism, and other aspects through both these 2 ways.[45,46] For example, miRNA-143 can promote angiogenesis and osteoblast differentiation *in vivo*[42]; miRNA-375 can regulate mammalian islet cell development and insulin secretion[43]; miRNA-181 can control mammalian hematopoietic stem cell differentiation,[47] and so on.

Numerous studies have revealed that a single mRNA may contain multiple binding sites complementary to multiple miRNAs, thus forming a complex regulatory network that participates in various biological mechanisms to regulate biological processes.[48] For example, circ-XPO1 regulates XPO1 expression by using miR-23a-3p, miR-23b-3p, miR-23c, and miR-130a-5p as competing endogenous RNAs (ceRNAs).[49] In addition, individual miRNAs can also target various mRNAs to exert regulatory effects on biological gene activity.[50] As a result of this complex regulatory network of miRNAs, one or more specific miRNAs can affect the expression of many downstream target genes through direct or indirect regulation and thus perform a wide range of biological functions.

In addition, miRNAs have been closely associated with human cancer development. Since the first evidence of the involvement of miRNAs in tumorigenesis was discovered in 2002,[51] for example, miRNA-372 and miRNA-373 in testicular germ cell tumors can neutralize p53-mediated CDK inhibition by directly inhibiting the expression of the tumor suppressor LATS2, thereby promoting tumorigenesis.[52] Furthermore, high expression of miRNA-421 was found in various tumors, including neuroblastoma, pancreatic cancer, and prostate cancer.[53–55] This suggests that miRNA-421 can act as a pro-

carcinogenic gene in human cancers.[56] However, miRNA-125a-5p can act as a tumor suppressor miRNA, and its regulatory mechanism is to regulate cell proliferation by regulating the cell cycle during the G1/S transition, thereby inhibiting the development of head and neck cancer.[57] The overexpression of miRNA-217 in hepatocellular carcinoma cells inhibits proliferation and migration and induces apoptosis.[58] These findings on the pro- or anticancer effects of miRNAs in tumor tissues may provide new ideas for clinical diagnosis, drug delivery, and targeted therapy.

The Role and Mechanism of miRNAs in the Development of Osteosarcoma Cells

The Role of miRNAs in the Proliferation of Osteosarcoma Cells

An in-depth study of the molecular mechanisms involved in the development of osteosarcoma is essential to improving the survival rate of patients with osteosarcoma.[59] The regulatory role of miRNAs in the development of osteosarcoma is diverse. Some miRNAs have been shown to act as oncogenes, others can act as a tumor suppressor by specifically regulating the expression of their target genes.[59] The regulatory role of miRNAs in the development of osteosarcoma is related to the activation status of various signaling pathways and the expression levels of some protein molecules.[60] Therefore, studying the signaling pathways including PI3K/AKT, PTEN/AKT, Wnt/β-catenin, Wnt, and NF-κB, and proteins including P21, IRF2, VEPH1, and BCL9L, may contribute to the development of osteosarcoma studies. We summarized the progress of miRNAs regulating osteosarcoma through these pathways (Figure 2).

miRNAs promote the proliferation of osteosarcoma cells. Various studies have shown that miRNAs play a role in developing osteosarcoma.[61] In this process, the role of miRNAs in promoting osteosarcoma proliferation is exerted through specific molecular mechanisms. Therefore, it is of great clinical value and significance to elucidate the regulatory mechanisms of miRNAs in this aspect of osteosarcoma. The regulatory mechanisms, upstream regulatory factors, and other malignant tumors with abnormal expression of some miRNAs that promote the progression of osteosarcoma are shown in Table 1.

P21 is involved in various cellular metabolic pathways, plays an essential role in promoting tumor proliferation, and is considered a promising target molecule in anticancer research.[81] The expression of miRNA-95-3p in osteosarcoma is significantly correlated with the clinical stage. The overexpression of miRNA-95-3p increases cell growth and inhibiteds apoptosis of osteosarcoma cells through TGF-beta/CDKN1A/p21/cyclin D1.[82] P21 has an extensive inhibiting effect on CDKs (cyclin-dependent kinases), especially on CDK2 and CDK4. The Cyclin-CDK complex can help cells quickly pass checkpoints of the cell cycles, and its overexpression can reduce cell mass and promote proliferation and transformation. P21waf1/cip1 plays a role in checkpoint control in inhibiting Cyclin-CDK.[83] The expression of CDKN1A/p21 is tightly controlled at the transcriptional level primarily by the tumor suppressor protein p53 in response to DNA damage.[83,84] In addition, the overexpression of miRNA-95-3p promotes tumor cell proliferation and migration in cultured cells and tumor growth in xenograft mouse models through negative posttranscriptional regulation of p21 by directly targeting the 3′-UTR.[85]

At the same time, miRNAs can also inhibit the development of osteosarcoma by targeting many proteins and molecules. For example, it has been found that miRNA-92a can promote the occurrence and development of hepatocellular carcinoma and cervical cancer expression.[86,87] In osteosarcoma, miRNA-92a can promote osteosarcoma cell growth and cell cycle progression by directly targeting and inhibiting FBXW7 while inhibiting apoptosis.[88] In addition, miRNA-92a can negatively regulate DKK3 expression and thus promote osteosarcoma cell progression.[89]

PI3K/AKT is another critical intracellular signaling pathway with essential regulatory functions in the cell cycle. This signaling pathway is activated in various tumors, including osteosarcoma.[90–92] The signaling pathway promotes proliferation, survival, and epithelial–mesenchymal transition (EMT) while suppressing apoptosis.[93] The carcinogenesis of miRNA-23b-3p in colon adenocarcinoma,[94] hepatocellular carcinoma,[95] and esophageal squamous cell carcinomas[96] has been confirmed. In osteosarcoma, miRNA-23b-3p can promote osteosarcoma by targeting VEPH1/PI3K/AKT signaling pathway.[97] MiRNA-23b-3p can also promote the apoptosis and inhibit the proliferation and invasion of osteosarcoma cells by targeting SIX1.[98] In another study, miRNA-18a-5p can promote the invasion and migration of OS cells by inhibiting IRF2 expression.[99] However, LncRNA FER1L4 can downregulate the expression of miRNA-18a-5p and block PI3K/AKT signaling pathway, effectively inhibiting the progress of OS.[100]

PTEN is a well-established tumor suppressor gene and is one of the most commonly mutated genes in various human cancers.[89] PTEN acts as a lipid phosphatase to dephosphorylate the 3′ position of phosphatidylinositol-34,5-trisphosphate(PIP3), the product of a potent proto-oncogenic phosphatidylinositide 3-kinase (PI3K), and triggers activation of the PI3K pathway. AKT kinase plays a key role in cell survival, cell proliferation, angiogenesis, and anabolism and is a major target for cancer therapy. One of the main targets for cancer therapy.[101] miRNA-21, miRNA-216, miRNA-155, and miRNA-524 can enhance the proliferation of osteosarcoma cells by targeting PTEN to activate the PI3K/Akt signaling pathway.[102–105] The PI3K/AKT signaling pathway is unregulated in most localized diseases and in 100% of advanced diseases, implying that alterations in this pathway may be a prerequisite for inhibiting osteosarcoma progression.[106]

Finally, the activation of the WNT/β-catenin signaling pathway has been highly conserved throughout evolution and plays a key role in regulating tissue development and maintaining homeostasis *in vivo*.[107] In human cancers, Wnt/β-catenin signaling is highly activated, such as cervical

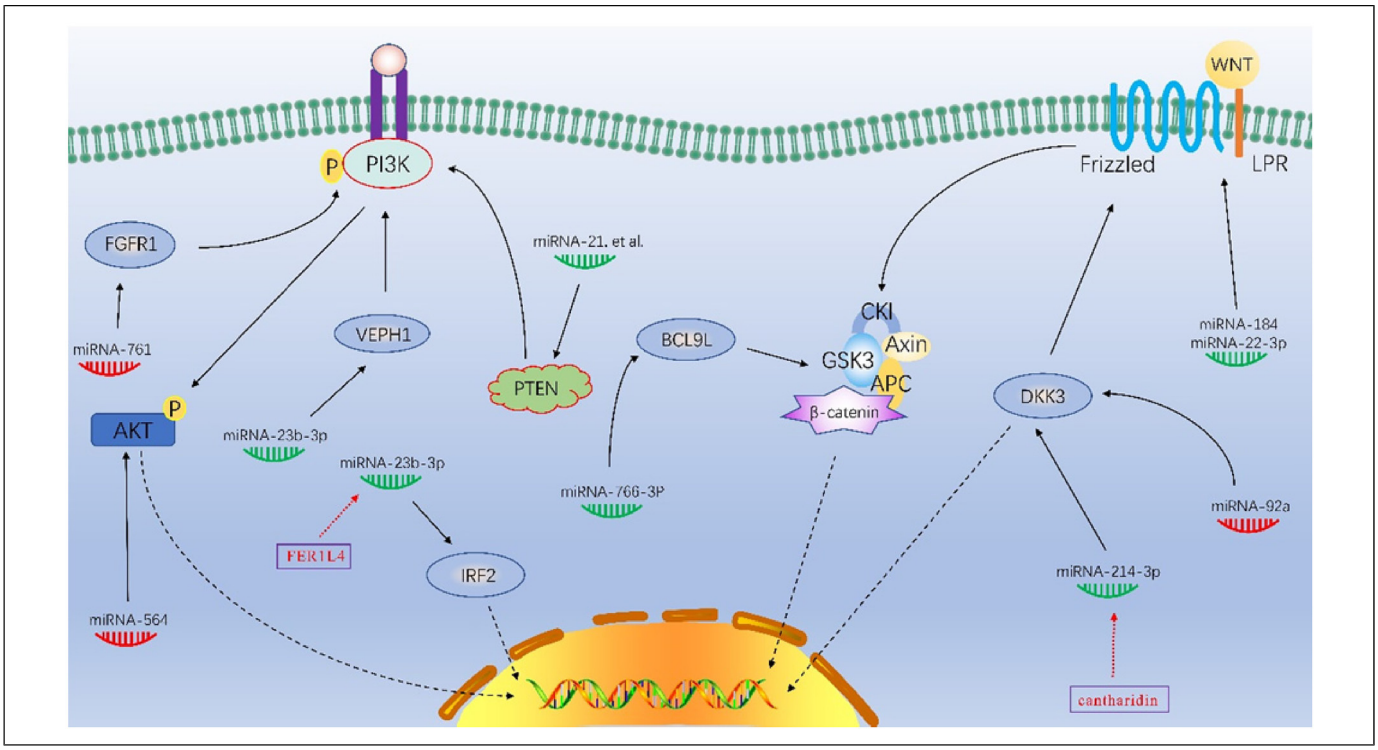

Figure 2. miRNAs can regulate PI3K/Akt and Wnt/β-catenin signaling pathways in many ways, thus affecting the progression of osteosarcoma (Green represents the promotion of osteosarcoma; Red represents the inhibition of osteosarcoma).

cancer,[108] nasopharyngeal carcinoma,[109] and gastric cancer.[110] And genetic and epigenetic deregulation of Wnt/β-catenin signaling contributes to human cancer, which has led to the development of extensive approaches targeting Wnt/β-catenin signaling as cancer therapies.[111] The recent research found that the inhibitory effect of baicalein,[112] alantolactone,[113] and schisandrin B[114] on osteosarcoma through Wnt/β-catenin signaling pathway. Some studies have found several miRNAs whose expression is specific to osteosarcoma, which can promote its occurrence and development by targeting Wnt/β-catenin signaling pathway. For example, miRNA-377-3p can inhibit osteosarcoma progression by targeting Cullin-1 (CUL1) and regulating Wnt/β-catenin signaling pathway.[115] In addition, miRNA-184 and miRNA-22-3p can also target this signaling pathway to promote the progress of osteosarcoma.[115,116] It has been studied that miRNA-214-3p can mediate Wnt/β-catenin/LEF1 signaling activation by targeting DKK3 to promote the oncogenesis of osteosarcoma, but this role of miRNA-214-3p is inhabited by cantharidin.[117] This indicates that cantharidin may be a prospective candidate for osteosarcoma treatment by targeting miRNA-214-3p/DKK3/β-catenin signaling pathways.

miRNAs inhibit the proliferation of osteosarcoma cells. In addition, a large number of studies have found many miRNAsare underexpressed in osteosarcoma.[61] These miRNAs can be amplified in the treatment of osteosarcoma by means of reducing the proliferation of osteosarcoma and thus inhibiting the tumor inhibition. By studying the targets and signaling pathways of these miRNAs, it may be possible to provide therapeutic targets for this purpose. The regulatory mechanisms, upstream regulatory factors, and other malignant tumors with abnormal expression of some miRNAs that inhibit the progression of osteosarcoma are shown in Table 2.

Astrocyte elevated gene-1 (AEG-1) is associated with various oncogenic signaling pathways such as Wnt and NF-kB, regulates tumor growth and metastasis, and plays an important role in osteosarcoma.[133] For example, miRNA-342-3p and miRNA-448 inhibit the progression of osteosarcoma by targeting AEG-1 to inhibit the Wnt and NF-κB signaling pathways.[134, 135] The NF-κB signaling pathway is responsible for cell proliferation, apoptosis prevention, and transcriptional regulation of various genes in response to damaging agents and cytokines and plays an important role in the pathogenesis of osteosarcoma.[136] For example, miRNA-29a inhibits the proliferation of osteosarcoma cells by negatively regulating its target DNMT3B, which inhibits the SOCS1/NF-κB signaling pathway.[137] Meanwhile, downregulation of miRNA-155 expression also inhibited osteosarcoma cell proliferation and induced apoptosis through the NF-κB signaling pathway.[138]

It has also been found that inhibition of PI3K/Akt and Wnt/β-catenin signaling pathways can also exert anti-osteosarcoma effects. For example, miRNA-761 inhibits the proliferation, migration and invasion of osteosarcoma cells and promotes apoptosis by directly targeting FGFR1 and inactivating the PI3K/Akt pathway. miRNA-564 downregulates osteosarcoma and inhibits the proliferation of osteosarcoma cells by targeting Akt.[139] In addition, miRNA-152 inhibites osteosarcoma cell proliferation by directly targeting the Wnt/β-catenin signaling

Table 1. miRNAs Which Are Downregulated in Osteosarcoma.

miRNAs	Gene targets	Signaling pathways	Effects	miRNA regulators	Other tumors
miRNA-4295[62]	PTPN14	YAP1	Restrict the growth and invasion of osteosarcoma cells	-	Gastric cancer↑[63] Head and neck squamous cell carcinoma↑[64]
miRNA-218[65]	Runx2	-	Inhibit proliferation, migration, and invasion of osteosarcoma cells	circEIF4G2[66] lncRNA SNHG10[67]	Glioma[68] Non-small cell lung cancer[69] Multiple myeloma[70]
miRNA-744-5p[71]	TGFB1	p38 MAPK	Suppress tumorigenesis and metastasis of osteosarcoma	-	Hepatocellular carcinoma[72] Glioblastoma[73]
miRNA-139[76]	ITGAV	-	Inhibit proliferation, migration, and invasion of osteosarcoma cell line MG63	LncRNA SNHG20[77] LncRNA LINC00858[78]	Multiple myeloma[74] Colorectal cancer[75] Pancreatic cancer[79] Prostate cancer[80]

Table 2. miRNAs Which Are Upregulated in Osteosarcoma.

miRNAs	Gene targets	Signaling pathways	Effects	miRNA regulators	Other tumors
miRNA-182-5p[118]	NKAPL	Notch	Regulate the cell cycle and promote proliferation of osteosarcoma	lncRNA SNHG10[119]	Bladder cancer[120] Hepatocellular carcinoma[121] Clear cell renal cell carcinoma[122]
miRNA-628-5p[123]	IFI44L	-	Promote the growth and movement of osteosarcoma	-	Cervical carcinoma[124] Colorectal cancer[125] Pancreatic ductal adenocarcinoma[126]
miRNA-4295[127]	IRF1	-	Promote proliferation, migration, and invasion of osteosarcoma	-	Gastric cancer[63] Head and neck squamous cell carcinoma[64]
miRNA-221[128]	FBXW11	Wnt	Promote cell proliferation and inhibit apoptosis in osteosarcoma	Lnc RNA GAS5[129]	Breast cancer[130] Thyroid cancer[131]
miRNA-221[132]	CDKN1B/ p27	-	Regulate proliferation, apoptosis, migration, and invasion of osteosarcoma		

pathway in a DKK1-dependent manner.[140] LncRNA PVT1 can be used as its upstream regulatory factor to enhance the chemical resistance of osteosarcoma.[141] However, LncRNA HAGLROS could promote osteosarcoma progression by sponging miRNA-152 to promote ROCK1 expression.[142] There are also some miRNAs that inhibit the progression of osteosarcoma, as shown in Table 1. Therefore, the discovery of these signaling pathways will help to enhance the role of these cancer-inhibiting miRNAs in osteosarcoma by using drugs or other auxiliary means in the future and provide a basis for the treatment of osteosarcoma.

The Role of miRNAs in Invasion and Metastasis of Osteosarcoma Cell

Metastasis is a complex process that describes the spread of cancer cells from the primary tumor site to other organs.[143] Metastasis is a complex multistep event and invasion of a tumor through the basement membrane into surrounding tissue is the first crucial step of the metastasis process. In other words, migration away from the primary tumor to adjacent tissues or organs, followed by migration of cancer cells through the stroma to blood vessels or lymphatic vessels to be transported to other organs where metastasis eventually occurs.[144] Metastasis is the hallmark of cancer and the leading cause of death in cancer patients.[145] In recent years, miRNAs have been reported to be involved in the invasion and metastasis of various tumors and have promised applications in many different cancers, including osteosarcoma.[59] Therefore, inhibiting osteosarcoma invasion and metastasis by selectively promoting and blocking fundamental molecular mechanisms associated with miRNAs may be a potential strategy for osteosarcoma treatment.

Similar to other cancer types, osteosarcoma cells undergo EMT in the early metastatic stages to facilitate their movement

and migration, and once osteosarcoma cells arrive and settle in a new metastatic site, such as the lung region, bone cancer cells, they can undergo EMT to allow them to adapt to and grow in their new environment.[146] Therefore, strategies that can prevent the development of early EMT osteosarcoma may help inhibit the spread of bone cancer. miRNA-486 is downregulated in many tumor cells. It acts as a tumor suppressor, regulating osteosarcoma cell invasion and EMT by targeting PIM1.[147] In addition, miRNA-766-3p can target BCL9L to inhibit EMT and metastasis of osteosarcoma through the β-catenin signaling pathway. It has also been shown that the miRNA-135b-TAZ positive feedback loop promotes EMT and migration and invasion of osteosarcoma cells and can also block metastasis of osteosarcoma cells by inhibiting TAZ protein expression and activity.[148]

In addition, several functional targets have been identified in osteosarcoma cells that bind to miRNAs, and miRNAs can inhibit osteosarcoma invasion and metastasis by binding to these targets. For example, E2F5 is a direct-binding target of miRNA-154-5p, and the overexpression of miRNA-154-5p can exert an inhibitory effect on osteosarcoma cell invasion and metastasis by inhibiting E2F5.[149] The expression of miRNA-25-3p is downregulated in osteosarcoma tissue samples and cell lines, and the overexpression of miRNA-25-3p could directly target SOX4 to inhibit osteosarcoma cell invasion and metastasis.[150] Besides, miRNA-92a inhibitors can inhibit osteosarcoma invasion and metastasis by upregulating DKK3 expression.[89] Downregulation of miRNA-4660 in osteosarcoma cells and reduction of the inhibition of MAFG expression by miRNA-4660 can also inhibit the invasion and metastasis of osteosarcoma cells.[151] By investigating and exploiting these potent targets of miRNAs, it may provide a strategy to inhibit osteosarcoma invasion and metastasis.

The Role of miRNAs in the Regulated Cell Death of Osteosarcoma

Selective elimination of cancer cells without damaging nonmalignant cells is essential in cancer treatment.[152] Unlike accidental cell death or an uncontrolled and passive process, regulated cell death can occur through a range of molecular mechanisms and signaling.[153] Therefore, rationally promoting regulated cell death in osteosarcoma cells may offer hope for osteosarcoma treatment. Regulated cell death can be further classified into subtypes based on the signaling and degradation pathways and the morphological characteristics of the biochemical endpoints of the dead cells.[153] Apoptosis was once thought to be the only form of regulated cell death. Still, recent studies have demonstrated that iron death and cell scorching are 2 other forms of regulated cell death.[154]

Apoptosis. Apoptosis is a regulated form of cell death that is triggered primarily by the activation of proteases of the cysteine aspartase family.[155] Apoptosis is a process of regulated cell death during normal development and aging. It is a mechanism for maintaining stable cell numbers.[156] It was found that miRNAs play an essential role in promoting apoptosis of osteosarcoma cells. MiRNA-204-5p, an oncogenic miRNA in osteosarcoma, promotes apoptosis and inhibits the migration of osteosarcoma via targeting EBF2.[157] Moreover, miRNA-497, for instance, can activate P21 expression by inhibiting the expression of MAPK/Erk signaling pathway and promoting the apoptosis of osteosarcoma cells.[158] MiRNAs have also been found to inhibit apoptosis in osteosarcoma cells. For example, miRNA-216 is an oncogene in osteosarcoma. miRNA-216 knockdown promotes apoptosis through PTEN/PI3K/AKT and related downstream genes P53 and MMP-2/9, thus exerting antitumor effects.[104] High expression of miRNA-1226-3p was associated with lower overall survival. It can inhibit the proliferation, migration, and invasion of osteosarcoma cells by targeting TRIAP3 and promote the apoptosis of osteosarcoma cells.[159] Successful treatment of osteosarcoma requires selective destruction of osteosarcoma cancer cells and attempting to selectively induce apoptosis in these osteosarcoma cells. It may offer hope for osteosarcoma treatment.

Ferroptosis. Despite the insight into the expression and function of some miRNAs in osteosarcoma, there are still many difficulties to be overcome by miRNAs inducing apoptosis in osteosarcoma cells and thus improving the treatment of osteosarcoma. Studies on ferroptosis in osteosarcoma may provide some effective ways to address these problems.[104,159,160] It plays a vital role in cancer and many degenerative diseases.[72,161] Ferroptosis was initially described as a non-apoptotic form of cell death characterized by impaired cellular uptake of cysteine, glutathione (GSH) depletion, and iron-dependent lipid peroxidation.[162] Ferroptosis differs morphologically and biochemically from apoptosis. For example, ferroptosis is accompanied by cell swelling and plasma membrane rupture. In contrast, apoptotic cells usually exhibit cell shrinkage and blistering of the plasma membrane.[163] With the discovery of various regulators and pathways, fundamental research into ferroptosis is rapidly expanding. Targeting ferroptosis may help develop novel therapeutic agents for treating malignancies offering new opportunities to treat various pathological conditions and diseases.[155,164] The mechanism of action related to ferroptosis in osteosarcoma is shown in.[165] Recently, it was also found that miRNA-1287-5p could inhibit the progression of osteosarcoma cells by inhibiting GPX4 to promote ferroptosis in osteosarcoma cells.[166] However, there are few literatures about the mechanism of miRNAs regulating ferroptosis in osteosarcoma, and a lot of research is needed to make up for this deficiency in the future.

Pyroptosis. More and more studies have recently found that pyroptosis can also be involved in the progression of osteosarcoma and may be a viable target for future osteosarcoma therapy.[167] The study also found that pyroptosis is an inflammatory form of cell death that releases specific cytokines and other immunostimulatory factors that protect mammals from

pathogens.[168] As a type of regulated cell death, pyroptosis is characterized by cell swelling, lysis, and the release of many pro-inflammatory factors.[169] Pyroptosis is also a highly immunogenic form of cell death, which can cause local inflammation and attract the infiltration of inflammatory cells, providing an excellent opportunity to alleviate the immunosuppression of the tumor microenvironment and induce the systemic immune response to treat solid tumors.[170] So pyroptosis can effectively eliminate malignant cells by specifically activating regulated cell death at their site of action, offering a new strategy for cancer treatment.[154] In osteosarcoma, it has been found that miRNA-181a can be aberrantly elevated in osteosarcoma tissues and cells. That knockdown of miRNA-181a can activate NLRP3-dependent pyroptosis to inhibit cell proliferation and invasion.[171] However, some studies have shown that pyroptosis is related to the negative effects of chemotherapy and radiotherapy. For example, chemotherapy and radiotherapy in tumor treatment may promote cell death of immune cells or hematopoietic cells, resulting in the damage of antitumor immune function.[170] Therefore, it is crucial to prevent normal cells from scorching induced by anticancer therapy to reduce the side effects of traditional therapy.

Overall, miRNAs regulate the occurrence and progression of osteosarcoma through their involvement in cell proliferation, invasion, metastasis, and apoptosis. These findings provide potential therapeutic targets and prognostic biomarkers for the involvement of miRNAs in the effective treatment of osteosarcoma. However, the specific mechanisms of action of many of these miRNAs are still unclear and need to be further investigated. Studies on the mechanisms of competitive endogenous RNAs(ceRNAs) have been continuously reported in recent years.[172] And more recently, the lncRNA–miRNA–mRNA axis,[173] circRNA–miRNA–mRNA regulatory networks,[174] etc, lay a theoretical foundation for in-depth study of the specific action mechanism of miRNAs, which can also provide better strategies for the diagnosis and treatment of osteosarcoma. Through continuous research on the role and mechanism of miRNAs in the development of osteosarcoma, we will guide the continuous improvement of the theoretical system of miRNAs, so that miRNAs can play a more significant role in clinical benefits.

The Potential Utility of miRNAs in Osteosarcoma

The Role of miRNAs in the Diagnosis and Prognosis of Osteosarcoma

Extracellular miRNAs(circulating miRNAs) can be stabilized by binding to proteins or encapsulated in vesicles, to be examined in biological fluids such as whole blood, plasma, and serum.[175] The high stability and ease of detection of miRNAs make them a good source of biomarkers for various diseases.[36] They are a good source of biomarkers for various diseases. Altered levels of circulating miRNAs are associated with tumor growth, progression, metastasis, and drug resistance, suggesting that they could potentially be used to optimize therapeutic approaches for tumor patients.[176] Analysis of circulating miRNA levels in patients' blood could therefore provide a new approach to the diagnosis or prognosis of cancer.

Existing studies have found that miRNA-139-5p expression in the serum of patients with osteosarcoma is significantly lower than that of healthy individuals and that miRNA-139-5p is more pronounced in patients with distant metastases or higher clinical stage.[177] In addition, increased serum levels of miRNA-542-3p are significantly associated with progressive tumor staging and shorter survival.[178] In addition, low plasma miRNA-375 levels are correlated with large tumor size, advanced clinical stages, positive distant metastasis, and poor tumor response to preoperative chemotherapy.[179] Other studies have found a significant reduction in serum miRNA-375 expression in osteosarcoma patients. Other studies have found that low serum miR-194 expression is strongly associated with positive metastasis, clinical stage, and poor survival and that serum miRNA-194 levels are significantly higher in osteosarcoma patients after surgery.[180] Therefore, detection of serum miRNA-139-5p, miRNA-542-3p, miRNA-375, and miRNA-194 expression can be used as reliable biomarkers to determine the diagnosis and prognosis of osteosarcoma.

As a promising biomarker, circulating miRNAs may open the door for diagnosing and prognosis of osteosarcoma and guide designing treatment plans and personalized surgical plans for osteosarcoma. However, the relatively difficult kinetic detection, low expression level, and short nucleotide sequence of circulating miRNAs challenge circulating miRNAs as a diagnostic and prognostic marker of osteosarcoma. Firstly, it must be clear that this difference in circulating miRNAs is not disease-specific. For example, it has been found that the expression of circulating miRNA-139-5p in osteosarcoma and lung cancer patients is downregulated.[177,181] Moreover, some drugs can target their targets and affect the expression of downstream miRNAs. For example, demethylating drugs such as 5-azacytidine (5-aza) can inhibit the proliferation and migration of hepatocellular carcinoma cells by upregulating the expression of miRNA-139-5p.[182] Therefore, the accuracy of circulating miRNAs will be interfered by diseases complicated with osteosarcoma or the use of some drugs. In addition, the low expression level of miRNAs in cells makes them exist less in biological fluids.[183] And miRNAs are sequenced with short base lengths, which lead to a high possibility of sequence similarity between homologous miRNAs.[183] So the potential application value of circulating miRNAs in osteosarcoma still needs further exploration.

The Role of miRNAs in Drug Resistance in Osteosarcoma

The standard treatment for patients with osteosarcoma includes chemotherapy for 10 weeks before surgery, surgical resection, and 20 weeks after surgery. Although there is some variation in chemotherapy regimens worldwide, the most commonly used regimens include cisplatin, doxorubicin, and high-dose methotrexate.[184] Long-term use of these chemotherapeutic

agents in patients with osteosarcoma tends to induce genetic mutations and resistance to the drugs, thus making osteosarcoma more difficult to treat.[10] In recent decades, treatment outcomes for osteosarcoma patients have not improved with newer chemotherapy drugs.[61] Many studies have found that miRNAs play an important role in improving drug resistance of osteosarcoma. Research in this area could provide a reliable and effective solution for future exploration of various related drugs and clinical treatment of osteosarcoma.[185]

miRNAs can be used as adjuvants in chemotherapy. Drug resistance remains a key challenge in current cancer chemotherapy, and miRNAs are strongly associated with changes in cancer chemosensitivity.[186] The use of miRNAs as chemotherapeutic adjuvants in the treatment of cancer would ameliorate this problem. For example, overexpression of miRNA-329-3p can also increase the sensitivity of osteosarcoma cells to cisplatin by targeting LDHA to inhibit glucose metabolism.[187] MiRNA-216b is significantly lower in osteosarcoma tissues than in paraneoplastic tissues, and increased miRNA-216b expression is associated with higher overall survival in osteosarcoma patients. Further studies revealed that JMJD2C is a target of miRNA-216b and that miRNA-216b could enhance cisplatin-induced apoptosis by regulating the JMJD2C/HIF1α/HES1 signaling axis in osteosarcoma cells.[188] Therefore, miRNA-216b can be used as a chemotherapeutic adjuvant than cisplatin to effectively treat osteosarcoma. In addition, low expression of miRNA-382 is closely associated with low survival rates in osteosarcoma patients. A related study found that overexpression of miRNA-382 could target and negatively regulate the expression of KLF12 and HIPK3, thereby enhancing the sensitivity of osteosarcoma cells to methotrexate and inhibiting the growth of osteosarcoma cells.[189] This resulted in enhanced sensitivity to methotrexate and inhibition of osteosarcoma cell growth. Increased miRNA-221 expression in osteosarcoma correlates with tumor staging, metastasis, and response to chemotherapy. miRNA-221 has been shown to induce resistance to adriamycin-induced apoptosis in osteosarcoma cells by activating the STAT3 signaling pathway and upregulating the expression of P-GP and BCL-2 proteins. STAT3-IN-3, a chemical inhibitor of the STAT3 signaling pathway, could effectively interfere with this effect and thus reverse the miRNA-221-induced resistance of osteosarcoma cells to adriamycin.[190] As the clinical treatment of osteosarcoma often uses a combination of chemotherapeutic agents, the development of multidrug resistance in osteosarcoma will seriously affect the efficacy of chemotherapy in osteosarcoma.[191] For example, SDC2 is a positive regulator of multidrug resistance in osteosarcoma. miRNA-20a-5p can regulate multidrug resistance to adriamycin and etoposide in osteosarcoma by directly acting on and inhibiting the target gene Syndecan-2(SDC2).[192] Overexpression of miRNA-20a-5p in osteosarcoma may help to overcome osteosarcoma drug resistance and be a candidate for preventing osteosarcoma chemoresistance.

miRNAs are involved in the regulation of autophagy in osteosarcoma. Autophagy is a cytoprotective mechanism that promotes cell survival by maintaining energy production under stressful conditions such as chemotherapy, hypoxia, and metabolic stress, maintaining intracellular homeostasis, development, differentiation, and processes essential for cell growth and survival.[193] However, in the presence of anticancer drugs, osteosarcoma cells can activate autophagy as a protective mechanism, making chemotherapy treatment of osteosarcoma difficult.[194] This would make chemotherapy for osteosarcoma difficult. Existing studies have shown that miRNAs can enhance chemoresistance or chemosensitivity through autophagy regulation in various tumors such as osteosarcoma, and inhibition of autophagy may be a new approach to improve the efficiency of chemotherapy in cancer treatment.[195] miRNA-22 can increase the sensitivity of osteosarcoma cells to cisplatin treatment by regulating autophagy-related genes. MTDH has been identified as a target of miRNA-22 in osteosarcoma cells and inhibition of MTDH-triggered autophagy by miRNA-22 plays a key role in the sensitivity to chemotherapy.[196] In addition, miRNA-22 is also found to play a key role in the sensitivity to chemotherapy. In addition, miRNA-410 is also found to enhance chemosensitivity by inhibiting autophagy in osteosarcoma cells. Apoptosis was significantly enhanced in cells treated with miRNA-410 combined with chemotherapeutic agents such as rapamycin, adriamycin, and cisplatin, compared to cells treated with chemotherapeutic agents alone.[197] Based on the above findings, miRNAs have an important role in enhancing the chemosensitivity of cells. However, finding effective inhibitors of autophagy remains a challenge. Further studies are needed to identify better and more appropriate inhibitors of autophagy to improve drug resistance in osteosarcoma.

The Application of miRNAs in Novel Nanomaterials

Despite tremendous advances in the treatment of osteosarcoma, significant challenges remain in terms of poor metabolism kinetics, high cytotoxicity, and drug resistance of drugs used in the treatment of osteosarcoma.[198] Developing new delivery mechanisms is another way to overcome resistance to conventional chemotherapeutic agents.[184] The use of drug-loaded nanomaterials with the ability to kill cancer cells and superior bioactivity is promising in the treatment of osteosarcoma. Nanotechnology may offer opportunities to overcome these difficulties by providing carriers for transporting molecules or other factors that affect gene expression in tumor cells.[198] For example, in one experiment, ZIF was used as a carrier to deliver miRNAs to achieve efficient cellular uptake and payload release at specific sites within the target cells. The results showed that the ZIF-8 vector exhibited high loading efficiency, promoted cellular uptake, and enhanced the endosomal escape of miRNAs. Both *in vitro* and *in vivo* evaluations also demonstrated that the miRNAs@ZIF-8 nanocomposite is a potential carrier for the effective delivery of therapeutic nucleic acid drugs.[199] In addition, miRNA-214 has been shown to playing an oncogenic role in osteosarcoma, promoting osteosarcoma invasion and migration, and mediating osteosarcoma drug resistance. miRNA-214 knockdown and

miRNA-214 inhibitors increase the radiosensitivity or chemosensitivity of osteosarcoma in osteosarcoma cells and xenograft mouse models.[200,201] Available studies have found that a highly positively charged nonviral vector (GO-PEI) complex can bind to negatively charged miRNA-214 and encapsulate miRNA-214 to synthesize miRNA-214 inhibitors, which can then be introduced into cells from *in vitro* or *in vivo* to exert tumor-suppressive effects. This inhibitor effectively inhibited the migration and invasion of MG63, and U2OS cells showed superior antitumor activity in an MG63 xenograft mouse model and enhanced cisplatin's killing effect on osteosarcoma cells.[202] Novel nanomaterials for drug delivery have the advantages of targeting specific cells for drug delivery, overcoming barriers to drug penetration, improving the bioavailability and therapeutic performance of antitumor drugs, and exploiting these advantages will offer hope for improving treatment strategies for osteosarcoma.

Summary and Outlook

In summary, this study reviewed the mechanism of miRNAs in the development of osteosarcoma and the application value of miRNAs in the diagnosis and drug resistance of osteosarcoma in recent years. The relationship between different miRNAs and patient prognosis, cancer diagnosis, cancer progression, chemotherapy resistance, and their application as possible drug targets has been demonstrated, and research on miRNAs in osteosarcoma development has achieved outstanding success. However, most miRNAs are still being studied *in vitro* studies of osteosarcoma cells. The specific mechanisms of many miRNAs in osteosarcoma, and genes and enzymes involved in the process of miRNAs acting on target genes, are still unknown. The accuracy of diagnostic and therapeutic target prediction needs to be improved. In addition, the literature on ferroptosis, pyroptosis, and other regulatory death pathways in osteosarcoma research is very limited and still needs much exploration by researchers. Although the differential expression of extracellular miRNAs has been repeatedly reported in osteosarcoma, due to the limitations of current detection methods and costs, no reliable miRNAs have been identified in the sera of osteosarcoma patients that can be used as diagnostic criteria for osteosarcoma, and the diagnosis and poor prognosis of osteosarcoma remains a serious problem for osteosarcoma patients. In addition, although miRNAs are becoming increasingly mature as early diagnostic indicators and combination chemotherapeutic agents in osteosarcoma, and relevant antagonistic drugs or gene mimetic drugs have been designed for cancer treatment in the course of therapy, the drug resistance mechanism of most osteosarcomas is still not clearly studied, and the research progress of miRNAs in drug resistance of osteosarcoma is still at a preliminary stage. In addition, the continuous development of nanomaterials will bring broader therapeutic prospects for osteosarcoma and may bring new therapeutic strategies to improve the problems of poor drug metabolism kinetics, high cytotoxicity, and drug resistance in osteosarcoma treatment. However, this aspect is limited by many factors, and the development is very slow and still needs much exploration by researchers.

In the research process, we found that miRNAs do not regulate the development of osteosarcoma through a single signaling pathway but through a complex network of signaling pathways, such as lncRNAs and miRNAs, circRNAs, and miRNAs, which together regulate the development of osteosarcoma. Therefore, future research on the role of miRNAs in the development of osteosarcoma should not be limited to the study of single miRNAs but should comprehensively evaluate and deeply explore these regulatory networks of miRNAs and identify common therapeutic targets from them. In addition, the combined diagnosis and treatment of osteosarcoma using one or more drugs that simultaneously target signaling pathways associated with different miRNAs may also amplify the effect of drugs in the treatment of osteosarcoma. This approach to identifying new targets for developing new therapeutic strategies and drugs may provide new ideas to improve these problems. Besides, the development and delivery of targeted therapeutic strategies using novel nanomaterials could be an effective treatment for diseases such as osteosarcoma, taking advantage of their therapeutic benefits. Osteosarcoma could be better treated by taking advantage of current developments and advances in technology.

In conclusion, there are still many challenges in the study of miRNAs in osteosarcoma. We believe that miRNAs will continue to be studied in depth in osteosarcoma in the future and will play an important role in the diagnosis, treatment, and prognosis of osteosarcoma. miRNAs are expected to be applied in the clinical treatment and diagnosis of osteosarcoma.

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