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REVIEW

Delivery of miRNAs Using Nanoparticles for the Treatment of Osteosarcoma

Chengran Wang[1,*], Yihong Zhang[1,*], Weihui Kong[2], Xin'ao Rong[1], Ziming Zhong[1], Lei Jiang[3], Shuhan Chen[1], Chuang Li[1], Fuqiang Zhang[1], Jinlan Jiang[1]

[1]Department of Scientific Research Center, China–Japan Union Hospital of Jilin University, Changchun, Jilin Province, People's Republic of China; [2]Department of Stomatology, the First Hospital of Jilin University, Changchun, Jilin Province, People's Republic of China; [3]Department of Geriatric Medicine, Changchun Central Hospital, Changchun, Jilin Province, People's Republic of China

*These authors contributed equally to this work

Correspondence: Fuqiang Zhang; Jinlan Jiang, Department of Scientific Research Center, China–Japan Union Hospital of Jilin University, Changchun, Jilin Province, People's Republic of China, Email zfqzhang@jlu.edu.cn; jiangjinlan@jlu.edu.cn

Abstract: Osteosarcoma is the predominant primary malignant bone tumor that poses a significant global health challenge. MicroRNAs (miRNAs) that regulate gene expression are associated with osteosarcoma pathogenesis. Thus, miRNAs are potential therapeutic targets for osteosarcoma. Nanoparticles, widely used for targeted drug delivery, facilitate miRNA-based osteosarcoma treatment. Numerous studies have focused on miRNA delivery using nanoparticles to inhibit the progress of osteosarcoma. Polymer-based, lipid-based, inorganic-based nanoparticles and extracellular vesicles were used to deliver miRNAs for the treatment of osteosarcoma. They can be modified to enhance drug loading and delivery capabilities. Also, miRNA delivery was combined with traditional therapies, for example chemotherapy, to treat osteosarcoma. Consequently, miRNA delivery offers promising therapeutic avenues for osteosarcoma, providing renewed hope for patients. This review emphasizes the studies utilizing nanoparticles for miRNA delivery in osteosarcoma treatment, then introduced and summarized the nanoparticles in detail. And it also discusses the prospects for clinical applications.

Keywords: osteosarcoma, MicroRNAs, nanoparticle delivery, molecular targeted therapy

Introduction

Osteosarcoma originates from primitive mesenchymal cells and is the predominant malignant primary bone tumor in children and adolescents aged 0–24 years.[1] Most osteosarcoma cases manifest in the lower long bones.[2] Osteosarcoma can be classified into the osteoblastic, chondroblastic, and fibroblastic subtypes.[3] A defining feature of osteosarcoma is the osteoid extracellular matrix, which is predominantly composed of collagen I.[4] Osteosarcoma is characterized by notable inter- and intra-tumoral heterogeneity.[5] The development of osteosarcoma is linked to intricate genetic mutations, notably chromothripsis, chromoplexies, and kataegis mutations.[6] The most frequently mutated genes involved in osteosarcoma pathogenesis are tumor suppressor p53 (TP53) and the retinoblastoma susceptibility gene (RB1). Additionally, approximately 90% of osteosarcomas exhibit mutations in breast cancer (BRCA)-related genes.[6] The recurrence and metastasis of osteosarcoma significantly affect patients' survival.[7] Consequently, novel osteosarcoma treatments with substantial research potential are urgently needed to target mutated genes.

Currently, the standard treatment for osteosarcoma is neoadjuvant chemotherapy supplemented by a combination of surgical and adjuvant therapies.[8] Osteosarcoma metastasis, influenced by the bone microenvironment, poses considerable challenges for both surgical and chemotherapeutic interventions.[9] Immunotherapy and targeted chemotherapy are emerging strategies for osteosarcoma treatment, encompassing the innovation of novel drug delivery systems.[10]

MiRNAs are single-stranded endogenous RNA, approximately 22 nucleotides in length.[11] Lee et al[12] first identified miRNAs in Caenorhabditis elegans in 1993. Within the nucleus, RNA polymerase II transcribes miRNA genes into pri-miRNAs, which

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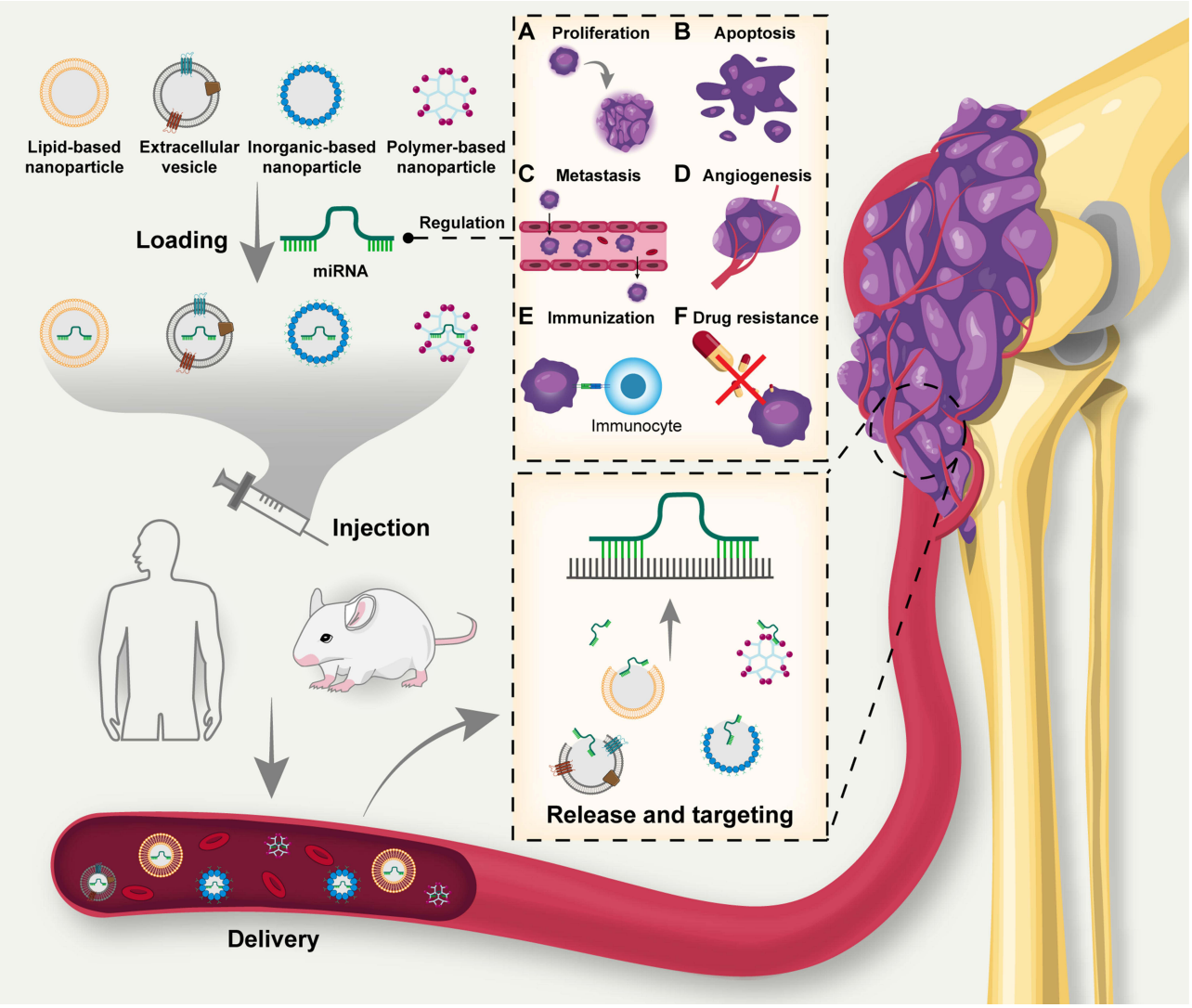
Graphical Abstract



are subsequently cleaved into pre-miRNAs by Drosha and its cofactor DGCR8/Pasha.[13] Subsequently, Exportin 5 transports pre-miRNAs to the cytoplasm, where Dicer cleaves them into small double-stranded RNAs termed miRNA duplexes.[14] The miRNA duplex is incorporated into the guide strand channel of an argonaute protein, resulting in the formation of an RNA-induced silencing complex (RISC).[15] The RISC complex then facilitates the recognition of the targeted mRNA, leading to either mRNA destabilization or translational repression (Figure 1).[16]

MiRNAs, functioning in association with argonaute and the 182 kDa glycine-tryptophan protein, engage with the mRNA 3′-untranslated region (UTR). This interaction leads to translational repression, deadenylation, and degradation, culminating in specific biological effects.[17] MiRNAs play pivotal roles in modulating diverse physiological processes, including proliferation, differentiation, and immunity.[18] Concurrently, miRNAs participate in the orchestration of cancer-associated pathological processes, including cell cycle regulation, proliferation, apoptosis, invasion, migration, and angiogenesis.[19,20] Therapies based on the gene-regulating functions of miRNAs have been used for tumor treatment.[21]

MiRNA delivery involves safeguarding and stabilizing endogenous or exogenous miRNA structures via diverse techniques. This process ensures targeted delivery to disease sites, facilitates gene regulation, and aids in disease

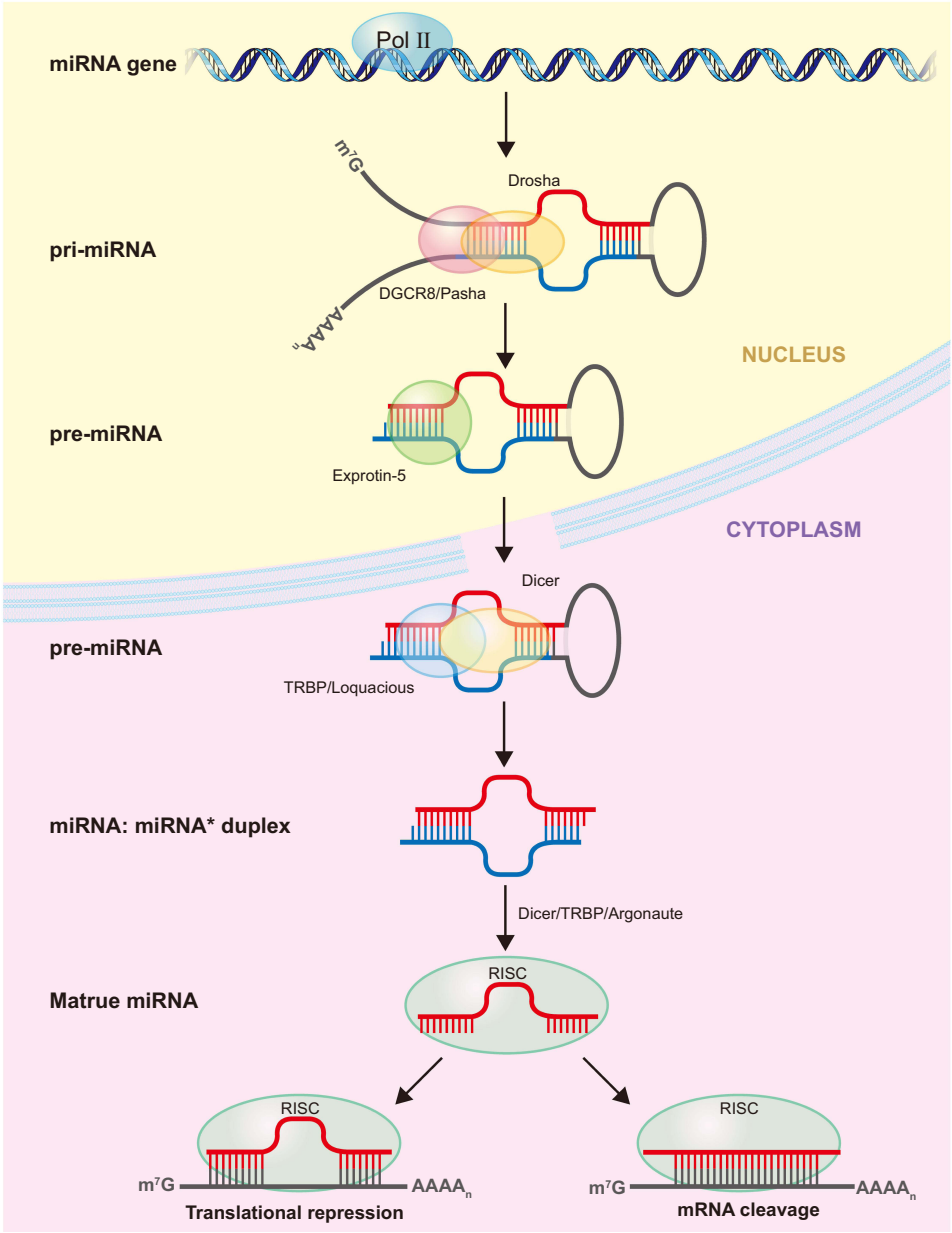


Figure 1 MiRNA Biogenesis: Typically, RNA polymerase II (Pol II) transcribes a miRNA gene into the primary miRNA (pri-miRNA). In the nucleus, the RNase III endonuclease Drosha, along with the double-stranded RNA-binding domain (dsRBD) protein DGCR8/Pasha, processes the pri-miRNA. This results in a 2-nt 3′overhang that encompasses the ~70-nt precursor miRNA (pre-miRNA). Exportin-5 transports the pre-miRNA into the cytoplasm. Here, another RNase III endonuclease, Dicer, in conjunction with the dsRBD protein TRBP/Loquacious, cleaves it. This action yields a 2-nt 3′ overhang that holds a ~21-nt miRNA:miRNA* duplex. The miRNA strand integrates into an Argonaute-containing RNA-induced silencing complex (RISC), through which it modulates mRNA translation.

treatment.[22] These miRNAs include tumor suppressors (TS miRNAs), oncogenes (oncomiRs), miRNA mimics, and molecules specifically targeting miRNAs (anti-miRs).[23] TS miRNAs, which are typically downregulated in cancer, and oncomiRs, which are often overexpressed in cancer, are endogenous.[24] Utilizing exogenous miRNAs to modulate the expression of endogenous miRNAs offers a therapeutic approach for cancer.

Encapsulation of miRNAs into nanocarriers can improve delivery efficiency and become a promising strategy for targeting therapy of cancer.[25] Currently, many types of vectors are used for delivery of miRNAs.[26] Polymer-based carriers are capable of adsorbing or encapsulating miRNAs and can also be modified with groups or molecules to increase performance.[27,28] Liposomes are highly biocompatible and have been widely used to deliver miRNAs.[29] Exosomes, secreted by cells, are capable of carrying various secretions and have homing properties to target lesions.[30]

Inorganic nanoparticle can be used in conjunction with a variety of methods, such as magnetic fields and photothermal therapy, to enhance the effect of miRNA delivery.[31]

Strategies centered on miRNA-targeted delivery for tumor treatment have demonstrated efficacy and have been transitioned into clinical trials, revealing significant application potential.[32] This advancement also kindles optimism for miRNA-based gene-targeted therapies for osteosarcoma. In this context, our study delves into the recent discoveries related to osteosarcoma treatment via miRNA delivery, summarizes the vectors used and explores the potentials for clinical applications.

Roles of miRNAs in Tumors

In 2002, Carlin et al[33] discovered that both miR-15 and miR-16 were either deleted or downregulated in 68% of patients with chronic lymphocytic leukemia, marking the inaugural identification of the association between miRNAs and tumors. Subsequently, researchers have progressively delved deeper into the relationship between miRNAs and tumors.[34,35] The dysregulation of expression of miRNAs in cancer is intricately linked to tumor pathogenesis.[36] Therefore, targeting and modulating miRNA expression may be a novel therapeutic approach for cancer treatment.[37] Deregulated miRNAs in pathological conditions can be addressed through miRNA replacement therapies using miRNA mimics or by inhibiting the function of miRNAs using anti-miRs, both of which hold therapeutic promise.[23] In 2013, MRX34, a synthetic double-stranded miR-34a mimic, was introduced clinically for the first time in tumor treatment.[38] A deeper exploration of the role of miRNAs in tumorigenic mechanisms could expedite their therapeutic application in tumor treatments.[39]

Multiple miRNAs play pivotal roles in the evolution and progression of breast and prostate cancers.[40] Xu et al[41] identified that miRNA-135 curbed the onset of epithelial-mesenchymal transition (EMT) in breast cancer. This was achieved by targeting and downregulating zinc finger protein 217 (ZNF217) and subsequently preventing Nanog homeobox (NANOG) upregulation by reducing N6-methyladenosine levels via methyltransferase-like 13 (METTL13). Gan et al[42] observed that elevated levels of miR-375 in patients with castration-resistant PCa could expedite prostate cancer progression and resistance to enzalutamide. This was achieved by disrupting the expression of phosphatase non-receptor type 4, which subsequently stabilized phosphorylated signal transducer and activator of transcription 3 (STAT3). Khan et al[43] determined that miR-1 directly targeted the 3'-UTR of CXC chemokine receptor type 4 (CXCR4), which hindered Forkhead box M1 (FOXM1) from binding to the Ribonucleotide reductase M2 (RRM2) promoter, thereby inhibiting the growth and metastasis of small cell lung cancer. Additionally, researches indicated that tumor-derived exosomes significantly influenced cancer development by promoting cancer proliferation, invasion, metastasis, EMT, and immune evasion.[44–46] Qiu et al[47] discovered that exo-miR-519a-3p derived from gastric cancer triggered M2 polarization in intrahepatic macrophages, leading to the establishment of premetastatic niches rich in angiogenesis. Zeng et al[48] discovered that exosomal miR-25-3p, originating from colorectal cancer cells, elevated the expression of vascular endothelial growth factor receptor-2 (VEGFR2), zonula occludens-1 (ZO-1), occludin, and Claudin5 in endothelial cells by targeting Krüppel-like factor 2 (KLF2) and Krüppel-like factor 4 (KLF4). This action enhanced vascular permeability and angiogenesis, thereby promoting colorectal cancer metastasis. Consequently, miRNAs are intricately linked to the biological behaviors of tumors and are promising targets for tumor therapy.[49]

Effects of miRNAs in the Occurrence and Development of Osteosarcoma

Numerous studies have explored the role of miRNAs in promoting osteosarcoma development, highlighting their potential therapeutic targets (Table 1).

Cell Proliferation

Cell proliferation is a defining characteristic of the cancer cells. Many studies explored the mechanisms by which miRNAs promote the proliferation of osteosarcoma cells.[69] The phosphatase and tensin homolog (PTEN) can curtail osteosarcoma proliferation by negatively modulating the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway.[70] A research indicated that miR-93 could target PTEN, leading to the inhibition of osteosarcoma proliferation.[50] In osteosarcoma cells, while miR-let-7a expression diminished, its target gene E2F Transcription Factor 2 (E2F2) was upregulated. Overexpression of let-7a in these cells markedly reduced tumor

Table 1 MiRNAs in Osteosarcoma

MiRNA	Effect on osteosarcoma	In vivo/in vitro trial	Proposed mechanism	References
MiR-93	Proliferation↑	In vivo and in vitro	PTEN↓	[50]
Let-7a	Proliferation↓	In vivo and in vitro	E2F2↑	[51]
	Metastasis↑	In vivo and in vitro	C15orf41↓	[52]
MiR-221	Proliferation↑	In vitro	FBXW11↓	[53]
	Apoptosis↓			
MiR-1281	Apoptosis↑	In vivo and in vitro	USP39↓	[54]
MiR-663a	Apoptosis↑	In vivo and in vitro	ZBTB7A↓	[55]
MiR-382	Metastasis↓	In vivo and in vitro	YB-1↓	[56]
MiR-101	Metastasis↓	In vivo and in vitro	BCL6↓	[57]
MiR-487b-3p	Metastasis↓	In vivo and in vitro	ALDH1A3↓	[58]
MiR-199a-5p	Angiogenesis↓	In vivo and in vitro	VEGFA↓	[59]
MiR-134	Angiogenesis↓	In vivo and in vitro	VEGFA↓, VEGFR1↓	[60]
MiR-381	Angiogenesis↓	In vivo and in vitro	VEGFA↓	[61]
MiR-CT3	Angiogenesis↓	In vitro	VEGFA↓	[62]
MiR-424-5p	Angiogenesis↓	In vitro	VEGFA↓	[63]
MiR-17/20a	Immune regulation (macrophages↓)	In vivo and in vitro	Csf1↓	[64]
MiR-499a	Drug resistance↓	In vivo and in vitro	SHKBP1↓	[65]
MiR-29b-1	Drug resistance↓	In vitro	(uncertified)	[66]
MiR-140	Drug resistance↑	In vitro	(uncertified)	[67]
MiR-215	Drug resistance↑	In vitro	DTL↓	[84]

growth in vivo.[51] Additionally, Zhang et al[53] discovered that overexpression of miR-221 in osteosarcoma cells bolstered osteosarcoma proliferation and induced apoptosis. This effect was attributed to the downregulation of F-box and WD repeat domain containing 11 (FBXW11), which amplified Wnt signaling activity.

Cell Apoptosis

Apoptosis, a structured and coordinated cell death process, is prevalent in both physiological and pathological states but is notably reduced in cancer.[71] Endoplasmic reticulum stress (ERS) arises from the accumulation of misfolded proteins within the ER, culminating in apoptosis of osteosarcoma cells.[72] Jiang et al[54] observed that during ERS, p53 upregulates miR-1281, which subsequently targeted and suppressed ubiquitin-specific protease 39 (USP39), thereby inducing apoptosis in osteosarcoma cells. Zhang et al[55] identified that in osteosarcoma cells subjected to tunicamycin or thapsigargin-induced ERS, miR-663a directly bound to ZBTB7A 3′-UTR, reducing its expression. This action negated ZBTB7A transcriptional repression of lncRNA GAS5, amplifying ERS-induced apoptosis.

Cell Metastasis

Metastasis involves the proliferation of cancer cells at sites distant from their origin and encompasses processes such as dissemination, dormancy, and colonization.[73] MiRNAs interact with multiple signaling pathways that regulate

osteosarcoma metastasis, presenting potential targets for metastasis inhibition and osteosarcoma treatment.[74] Osteosarcoma cells can lose polarity and initiate invasion and migration via EMT, a process modulated by miRNAs.[75] Xu et al[56] observed the downregulation of miR-382 in osteosarcoma cells. However, elevating miR-382 levels curtailed EMT and osteosarcoma cell metastasis by targeting Y box-binding protein 1. Zhang et al[57] observed that miR-101 expression was markedly reduced in metastatic osteosarcoma cells compared with non-metastatic cells. However, amplifying miR-101 expression hindered osteosarcoma cell invasion and migration by suppressing the expression of B-cell lymphoma 6 (BCL6), an osteosarcoma tumor suppressor. Cheng et al[58] discovered that elevated miR-487b-3p levels suppressed osteosarcoma cell migration by targeting aldehyde dehydrogenase family 1 member 3 (ALDH1A3).

Angiogenesis

Angiogenesis is the process through which new capillaries emerge from an existing vascular network.[76] The vascular endothelial growth factor (VEGF) plays a pivotal role in regulating angiogenesis.[77] VEGF facilitates the detachment of pericytes from the basement membrane, weakens the extracellular matrix via proteolytic degradation, and subsequently drives the migration and proliferation of the endothelial cells lining the inner walls of blood vessels.[78] VEGF promotes the proliferation and survival of endothelial cells and contributes to tumor angiogenesis.[79] Thus, targeting VEGF could be a strategy for curtailing tumor growth.

Emerging evidence indicates that miRNAs play a pivotal role in regulating tumor angiogenesis.[80] Numerous studies have demonstrated that miRNAs modulate osteosarcoma angiogenesis by targeting VEGF-A. Zhang et al[59] discovered that exosomal miR-199a-5p derived from osteosarcoma cells translocated to human umbilical vein endothelial cells (HUVECs), targeting and suppressing VEGFA expression, thereby inhibiting osteosarcoma growth and angiogenesis. Zhang et al[60] observed that miR-134 expression was reduced in osteosarcoma cells. However, when overexpressed, miR-134 targeted and suppressed VEGFA and VEGFR1, thereby inhibiting osteosarcoma angiogenesis and proliferation. Tsai et al[61] discovered that Wnt-induced signaling protein 1 (WISP-1) enhanced VEGFA expression and angiogenesis by diminishing miR-381 expression, thereby advancing osteosarcoma progression. Raimondi et al[62] identified that miR-CT3 curtailed tumor angiogenesis by targeting VEGFA. Moreover, anti-angiogenic medications can suppress VEGFA synthesis through miRNAs, consequently inhibiting angiogenesis in osteosarcoma. Vimalraj et al[63] revealed that melatonin elevated miR-424-5p expression in osteosarcoma, leading to VEGFA inhibition.

Immune Regulation

The tumor microenvironment (TME) comprises a network of immune cells that secrete numerous cytokines, regulating immune responses and influencing tumor behavior.[81] Macrophages are pivotal components of the TME. Nirala et al[64] determined that hyperactivation of myelocytomatosis oncogene (MYC) resulted in macrophage colony-stimulating factor 1 (CSF1) downregulation due to elevated miR-17/20a expression, leading to reduced macrophage presence in the osteosarcoma TME. Yan et al[52] observed the upregulation of let-7a levels in exosomes derived from tumor-associated macrophages (TAMs). This upregulation targeted the 3'-UTR of C15orf41, leading to increased invasion and migration in osteosarcoma.

Drug Resistance

Chemotherapy resistance poses a significant challenge to the treatment of osteosarcoma. However, strategies targeting the genes responsible for this resistance are promising.[82] MiRNAs can target signaling pathways associated with chemoresistance genes in osteosarcoma, thereby enhancing the sensitivity of cells to chemotherapy.[83] Wang et al[65] discovered that transforming growth factor-β (TGFβ)-induced EMT reduced miR-499a expression via Snail1/Zeb1 binding directly to the miR-499a promoter. This subsequently elevated SH3KBP1-binding protein 1 (SHKBP1) expression, a target of miR-499a, thereby increasing erlotinib resistance in osteosarcoma cells. Di Fiore et al[66] observed the downregulation of miR-29b-1 in human osteosarcoma cells. However, miR-29b-1 upregulation suppressed stemness and increased chemosensitivity in the 3AB-osteosarcoma cancer stem cell (CSC) human osteosarcoma cell line. Song et al[67] revealed that miR-140 enhanced chemoresistance in osteosarcoma cells by targeting histone deacetylase 4. Song et al[84] determined that miR-215 induced G2 phase blockade by targeting and suppressing denticleless E3 ubiquitin protein ligase homolog (DTL) expression, thereby reducing cell proliferation. This resulted in the increased resistance of osteosarcoma cells to methotrexate and Tomudex.

MiRNA Delivery

MiRNAs that are intricately linked to the signaling pathways involved in the onset and progression of osteosarcoma can be modulated to hinder osteosarcoma progression.[68] While miRNA-based therapies hold significant promise for tumor treatment, the direct introduction of miRNAs as drugs into the body can substantially reduce their efficacy.[85] The direct introduction of exogenous miRNA mimics and anti-miRs into the body poses challenges, including degradation by RNases, potential absorption by tissues and organs, and possible harm to the organism.[86] Consequently, drug delivery systems that safeguard miRNAs, ensure targeted delivery, enhance their efficacy, and accelerate their clinical application, are needed.

Currently, vectors for miRNA delivery for tumor treatment are categorized into viral and nonviral types.[87,88] While viral vectors have a high delivery efficiency, they pose significant safety concerns. In contrast, nonviral vectors, despite their lower delivery efficiency, offer greater safety and hold substantial promise for clinical use.[89] Consequently, recent clinical studies on miRNA delivery vectors have predominantly focused on nonviral vectors.[90]

Treatment of Osteosarcoma by miRNA Delivery

Using nanoparticles to deliver miRNAs for cancer treatment has proven to be feasible.[91] Moreover, miRNA delivery can be used to treat osteosarcomas. In this section, we discuss studies on the use of nanomaterials for miRNA delivery in osteosarcoma treatment (Figure 2 and Table 2).

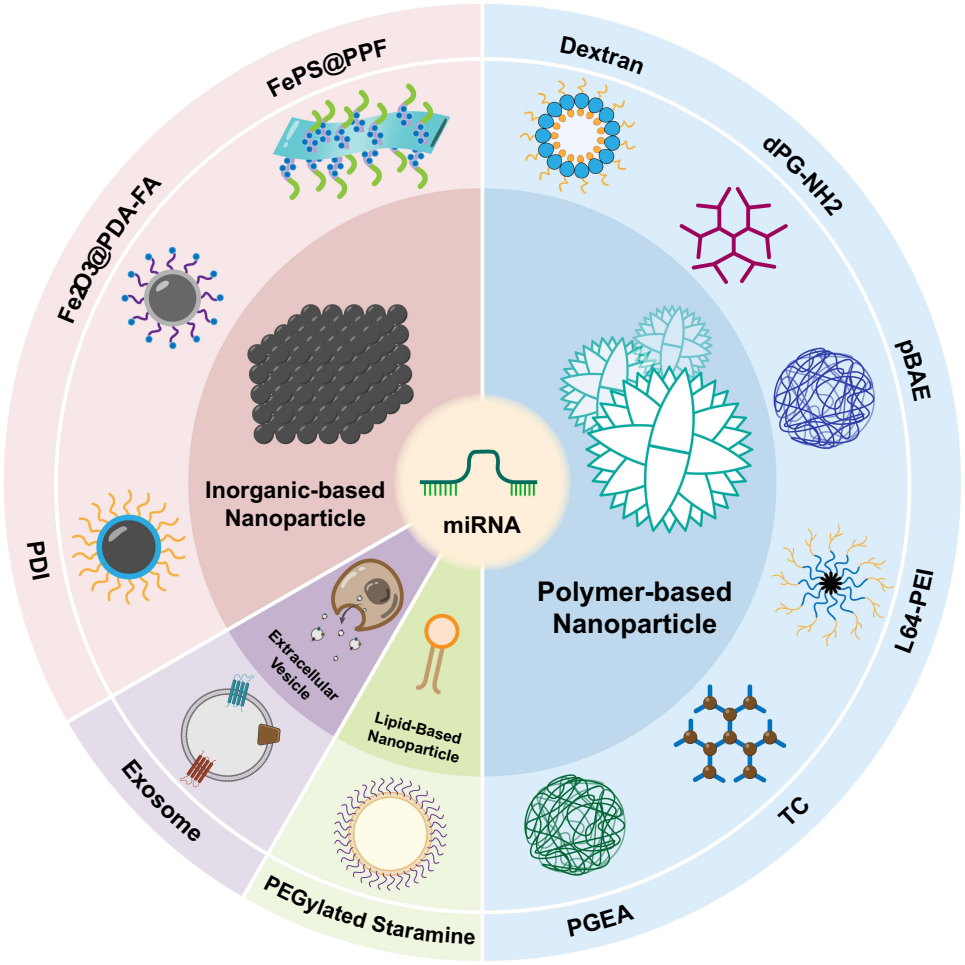


Figure 2 Nanoparticles for miRNA Delivery: Historical studies have categorized nanoparticles used for miRNA delivery in osteosarcoma treatment into four main types. The first category encompasses polymer-based nanoparticles, which include dextran, dPG-NH_2, pBAE, L64-PEI, TC, and PGEA. The second category includes lipid-based nanoparticle, containing PEGylated Staramine nanoparticle.The third category involves extracellular vesicles, notably exosomes. The fourth category comprises inorganic-based nanoparticles, including PDI, Fe_2O_3@PDA-FA, and FePS@PPF.

Table 2 Nanoparticles for miRNA Delivery

Nanoparticle type	Composition	MiRNA element	Drug co-delivery	In vivo/in vitro trial	Size [nm]	Reference
Polymer-based	Dextran	MiR-199a-3p, let-7a	–	In vitro	351.6±2.5	[92]
	Dendritic polyglycerolamine (dPG-NH_2)	miR-34a Mimics, miR-93 mimics, miR-200c mimics	–	In vivo and in vitro	80.32±23.79	[93]
	Poly-beta-amino-esters (pBAE)	MiR-29b mimics	Doxorubicin	In vivo and in vitro	151±2	[94]
	Pluronic® L64-polyethyleneimine (L64-PEI)	MiR-145	–	In vitro	≈100-250	[95]
	TGIC-CA (TC)	MiR-22	Volasertib	In vivo and in vitro	≈200	[96]
	Ethanolamine (EA)-modified poly (glycidyl methacrylate) (PGEA)	MiR-223	–	In vivo and in vitro	≈180	[97]
Lipid-based	PEGylated Staramine nanoparticles	Anti-miR-20a oligonucleotides	–	In vivo and in vitro	–	[98]
Extracellular vesicles	BMSCs-derived exosomes	MiR-206 mimic	–	In vivo and in vitro	≈100	[99]
	AD-MSCs-derived exosomes	The lentiviral particles encoding miR-101	–	In vivo and in vitro	–	[57]
	MSCs-derived exosomes	MiR-22 mimics	–	In vitro	–	[100]
	BMSCs-derived exosomes	MiR-143	–	In vitro	60–180	[101]
	H143B-derived exosomes	The lentiviral particles encoding miR-144-3p	–	In vivo and in vitro	131.8	[102]
Inorganic-based	Poly(ethylenimine)-dextran-iron oxide nanoparticles (PDIs)	MiR-302b plasmids	–	In vivo and in vitro	148.67 ± 1.52	[103]
	Polydopamine (PDA) -coated, folate (FA) -modified iron oxide (Fe_2O_3@PDA-FA)	NH_2-miR-520a-3p	–	In vivo and in vitro	89 ± 2.08	[104]
	$FePS_3$ and poly-L-lysine-PEG-folic acid (FePS@PPF)	Anti-miR-19a	–	In vivo and in vitro	206.1	[196]

Abbreviations: miRNAs, microRNAs; TP53, tumor suppressor p53; RB1, retinoblastoma susceptibility gene; BRCA, breast cancer; RISC, RNA-induced silencing complex; UTR, untranslated region; TS, tumor suppressors; EMT, epithelial-mesenchymal transition; ZNF217, zinc finger protein 217; NANOG, Nanog homeobox; METTL13, methyltransferase-like 13; STAT3, signal transducer and activator of transcription 3; CXCR4, CXC chemokine receptor type 4; FOXM1, Forkhead box M1; RRM2, Ribonucleotide reductase M2; VEGFR2, vascular endothelial growth factor receptor-2; ZO-1, zonula occludens-1; KLF2, Krüppel-like factor 2; KLF4, Krüppel-like factor 4; PTEN, phosphatase and tensin homolog; PI3K/AKT/mTOR, phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin; E2F2, E2F Transcription Factor 2; FBXW11, F-box and WD repeat domain containing 11; ERS, endoplasmic reticulum stress; USP39, ubiquitin-specific protease 39; BCL6, B-cell lymphoma 6; ALDH1A3, aldehyde dehydrogenase family 1 member 3; VEGF, vascular endothelial growth factor; HUVECs, human umbilical vein endothelial cells; WISP-1, Wnt-induced signaling protein 1; TME, tumor microenvironment; MYC, myelocytomatosis oncogene; CSF1, colony-stimulating factor 1; TAMs, tumor-associated macrophages; TGFβ, transforming growth factor-β; SHKBP1, SH3KBP1-binding protein 1; CSC, cancer stem cell; DTL, denticleless E3 ubiquitin protein ligase homolog; PEG, polyethylene glycol; dPG-NH_2, dendritic polyglycerolamine; pBAE, poly-beta-amino esters; HA, hyaluronic acid; CD44, cluster of differentiation-44; Dox, doxorubicin; PEI, polyethyleneimine; TGIC, 1,3,5-triglycidyl isocyanurate; CA, cystamine; PGEA, ethanolamine-modified poly (glycidyl methacrylate); MPS, mononuclear phagocyte system; MSCs, mesenchymal stem cells; EVs, extracellular vesicles; MSC-EVs, mesenchymal stem cell-extracellular vesicles; hBMSC, human bone marrow-derived mesenchymal stem cell; TRA2B, transformer 2 protein homolog beta; AD-MSC, adipose-derived mesenchymal stem cell; MNPs, magnetic nanoparticles; PDIs, poly (ethylenimine), dextran, and iron oxide nanoparticles; YOD1, YOD1 deubiquitinase; Chi-xPEI, chitosan-PEI crosslinked polymer; PTT, photothermal therapy; PDA, polydopamine; Fe_2O_3@PDA-FA, polydopamine-coated, folate-modified iron oxide; PPF, poly-L-lysine-PEG-folic acid; FePS@PPF, a multifunctional nanoplatform crafted from $FePS_3$ and poly-L-lysine-PEG-folic acid; NIR, near-infrared.

MiRNA Delivery via Polymer-Based Nanoparticles

Dextran is a water-soluble, biocompatible, and biodegradable substance that is non-toxic and non-immunogenic. Its properties can be enhanced by structural modifications, making it a popular drug delivery system.[106] Zhang et al[92] used lipid-modified dextran-based polymeric nanoparticles to introduce miR-199a-3p and let-7a into osteosarcoma cells,

inhibiting proliferation and growth of osteosarcoma cells. The lipid-modified dextran is amphiphilic dextran copolymers, which can self-assemble in an aqueous environment by hydrophobic interactions, forming spherical nanoparticles known as micelles.[107] The drug can then be encapsulated in a hydrophobic environment inside the carrier, increasing stability of the drug.[108] Also, the polyethylene glycol (PEG) on its surface reduces the clearance of the carrier by the reticuloendothelial system, thereby increasing the retention time of the drug in the circulation.[109] Zhang et al was the first to use polymers to encapsulate miRNAs to inhibit osteosarcoma. The effectivity and targetability of lipid-modified dextran-based polymeric nanoparticles to the osteosarcoma within organisms are uncertain because Zhang et al did not conduct vivo experiments in osteosarcoma. In addition, the safety of the lipid-modified dextran-based polymeric nanoparticles on organisms is still needed to verify by more experiments.

Dendritic polyglycerolamine (dPG-NH_2) is a nanocarrier derived from polyglycerol. dPG-NH_2 possesses 175 amines per mole of polymer, enabling it to bind negatively charged miRNAs.[93] Tiram et al[93] successfully delivered miR-93,[50] miR-200c,[110] and miR-34a[111] into human osteosarcoma cells both in vitro and in vivo by dPG-NH_2. These miRNAs induced tumor dormancy which extending time window of treatment.[93] Therefore, miRNA delivery not only inhibits osteosarcoma growth, but also brings hopes for the applications of other treatments of osteosarcoma. And it inspires the idea that we could combine other treatments, such as chemotherapy and surgery, with miRNA delivery to increase the therapeutic effect. Additionally, the enhanced permeability and retention effect allows nanoparticles to penetrate and accumulate more effectively in tumor cells, thus enhancing the therapeutic impact of drugs encapsulated within the nanoparticles.[112,113] Tiram et al[93] demonstrated the accumulation of miRNAs in tumor cells through in vivo experiments, which suggested that dPG-NH_2 successfully delivered miRNAs into the tumor tissue. But we do not know the distribution of miRNAs in other tissues and organs in the body compared to tumor tissues, which is related to the vector's tropism to tumors. One study highlighted that cationic nanoparticles disrupted cell membrane integrity more significantly than anionic nanoparticles, posing a potential biological risk.[114] Hence, experiments are also needed to explore the biotoxicity of dPG-NH_2.

Poly-beta-amino esters (pBAE) are cationic polymers synthesized through the Michael addition of acrylates to amines.[115] pBAE has been extensively used in drug delivery research because of its biocompatibility, biodegradability.[116] Freeman et al[94] used pBAE combined with hyaluronic acid (HA) to deliver miR-29b for osteosarcoma treatment. As the HA underwent swelling and degradation, it gradually released pBAE to deliver miRNAs progressively,[94] which contributed to reduce the number of administrations and increase patient compliance in clinical therapy. In addition, the receptor for HA, cluster of differentiation-44 (CD44), which is upregulated in OS cell lines,[117] is overexpressed in various cancers and is used for CD44-mediated tumor targeting.[118] Therefore, the aggregation of nanoparticles and miRNAs within osteosarcoma cells under HA targeted delivery in vivo can be further investigated in the future. Additionally, Freeman et al[94] combined systemic doxorubicin (Dox) administration with the targeted delivery of miR-29b. They demonstrated that miR-29b offered benefits in terms of reduced osteolysis which resulted from Dox.[94] Thus, delivery of miRNAs not only increases the efficacy of chemotherapeutic drugs, but also alleviates the suffering of body caused by chemotherapy. Furthermore, the above studies demonstrated that miRNA-targeted delivery combined with chemotherapeutic agents was effective and feasible.

Magalhães et al[95] employed a micellar nanosystem composed of the amphiphilic copolymer Pluronic® L64 and the cationic polymer polyethyleneimine (PEI) for the delivery of therapeutic miRNA-145 to osteosarcoma cells. PEI is a cationic polymer with several positive charges.[119] Conversely, RNA is a biomolecule with a prominent negative charge.[120] It can bind to PEI, facilitating its passage through negatively charged cell membranes.[121] Pluronic® L64, a neutral amphiphilic triblock copolymer, has a hydrophobic core and a hydrophilic shell, which can interact with phospholipid pairs in cell membranes and alter the membrane structure and function.[122] Chen et al[122] discovered that in a cellular internalization assay of PEI/pDNA, L64 enhanced the permeability of endosomal/lysosomal membranes, facilitating the escape of PEI/pDNA from endosomal/lysosomal catabolism. So Pluronic® L64 serves as a guardian, increasing the retention time of miRNAs in the circulation to serve as an effective vector for miRNA delivery. miR-145, which is diminished in osteosarcoma tissues and cell lines, inhibits osteosarcoma cell proliferation and invasion by targeting and silencing Rho-associated protein kinase 1.[123] It also targets VEGF to inhibit osteosarcoma cell invasion and angiogenesis.[124] Additionally, miR-145 suppresses EMT in osteosarcoma by targeting Snail, a potent repressor of

E-cadherin transcription.[125] In an in vitro study using the osteosarcoma cell line MG-63, Magalhães et al[95] demonstrated that the L64-PEI/miR-145 drug formulation effectively transported and released miR-145 into the cell cytoplasm which led to apoptosis and hindered cell migration. But PEI, due to its numerous amine groups, has evident cytotoxicity to cells.[126,127] However, as mentioned earlier, PEI readily binds to RNA and serves to act as a transporter or bridge to other substances and it is an excellent carrier for RNA delivery. Therefore, we can modify PEI to reduce its toxicity.[128,129] Pluronic® L64 has been studied for the use of intramuscular delivery of genes.[130,131] But the study on Pluronic® L64 in tumor therapy is not sufficient. Thus, its toxicity in living organisms still needs to be further investigated.

Chen et al[96] employed TGIC-CA (TC), a hydroxyl-rich, reduction-responsive cationic polymeric nanoparticle. It was synthesized via a one-step epoxy ring-opening reaction between 1,3,5-triglycidyl isocyanurate (TGIC) and cystamine (CA) to deliver miRNA-22 for osteosarcoma treatment.[96] Owing to its abundant hydroxyl groups, this polyhydroxy cationic polymer, synthesized using reagents with multiple amino or epoxy groups through a direct ring-opening reaction, exhibited higher transfection efficiency and lower cytotoxicity than PEI.[126] Additionally, Volasertib, a potent cell cycle kinase inhibitor, was delivered using TC along with miR-22.[96] Research has indicated that both miR-22 and Volasertib inhibit the PI3K/Akt pathway.[132,133] The combined therapeutic effect of TC/miR-22 and Volasertib was proved to be better than that of TC/miR-22 alone.[96] Hence, combining miRNA-targeted delivery of polymer with chemotherapeutic agents holds significant promise for osteosarcoma treatment.

Ethanolamine-modified poly (glycidyl methacrylate) (PGEA) is a cation carrier enriched with hydroxyl groups. It has low toxicity and high transfection efficiency, making it a popular choice in recent gene therapy studies.[134,135] Research has indicated that miR-223 plays a role in hindering the progression of osteosarcoma.[136,137] Chen et al[97] used PGEA to deliver miR-223 in vivo, which effectively inhibited the proliferation, invasion, and migration of osteosarcoma cells without causing notable toxicity. Additionally, the adjacent nonionic hydrophilic hydroxyl groups boost the affinity of the cationic polymer for the anionic gene, enhancing transfection efficiency and also shielding the potentially harmful cationic charge of the carrier.[138,139] The presence of hydroxyl groups diminishes the interaction between PGEA and serum proteins, thereby promoting its stability in the bloodstream.[140–143] Hence, the introduction of hydroxyl group modifications significantly enhances cationic polymer carriers' affinity for miRNAs and reduces the probability of being disturbed by substances in the blood circulation.

The in vivo metabolism, accumulation, and side effects of polymer vectors also need to be considered if polymer vectors are to be used in the clinic for delivery of miRNAs for the treatment of osteosarcoma.[144–146]

MiRNA Delivery via Lipid-Based Nanoparticles

Liposomes have been widely studied for in vivo drug delivery.[147] PEGylation shields nanocapsules from plasma protein adsorption and mononuclear phagocyte system (MPS) detection, thereby facilitating their circulation in the bloodstream and subsequent drug release.[148] Liposomes with a particle size of 20–100 nm were reported to exhibit uniform drug encapsulation, stable drug release, and longer circulation times.[149] And the particle size of PEGylated Staramine was about 80–100 nm according to a previous research.[150] Yang et al[98] used PEGylated Staramine to deliver anti-miR-20a oligonucleotides targeting Fas to suppress lung metastatic osteosarcoma by intravenous injection. Therefore, to address the early metastatic nature of osteosarcoma, systemic administration, for example, intravenous injection, may plays a more effective role in inhibiting potential osteosarcoma metastases.[151] However, research has indicated that PEGylated lipid nanocarriers possess immunogenic properties, potentially diminishing carrier absorption and posing safety concerns.[152] However, subsequent research showed that polyethylene glycosylation did not increase cell death rates.[153] And perhaps the formulation of different kinds of lipids affects the cytotoxicity of lipid nanoparticles.[153,154] In addition, application of liposomes with excessive diameter should be avoided as it may lead to fat embolization.[155,156] And whether the immune evasion of liposomes for a long period of time will have adverse effects on the organism still needs to be further investigated.

MiRNA Delivery via Extracellular Vesicles

Mesenchymal stem cells (MSCs) are pluripotent cells with the ability to differentiate into diverse cell types and exhibit immunomodulatory and tumor-homing properties.[157] Given their low immunogenicity and tumor-homing capabilities,

MSCs can be engineered to produce antitumorigenic miRNAs.[158] Exosomes are extracellular vesicles (EVs) measuring 40–160 nm in diameter that carry substances from their originating cells. They are secreted and play a role in intercellular signaling.[159] Mesenchymal stem cell-extracellular vesicles (MSC-EVs) exhibit low immunogenicity and possess tumor-targeting capabilities, making them suitable for the delivery of miRNAs in tumor treatment.[160] Numerous studies have explored miRNA delivery using MSC-EVs.[161]

MSC-EVs significantly influence the onset, progression, and treatment of osteosarcoma.[162] However, exosomes from original MSCs may facilitate progression of cancer.[163] That is because primitive MSCs are able to secrete exosomes carrying miRNAs which can promote proliferation of osteosarcoma.[164–166] Thus, engineered exosomes from MSCs are ideal miRNA carriers for targeted tumor therapy.[167] Given that certain MSC-EVs can enhance tumor growth, employing exosomes from MSCs with tumor-suppressive traits can be an effective strategy for cancer therapy.[168]

Numerous studies have focused on engineered MSC exosomes carrying miRNAs targeting osteosarcoma and their specific mechanism. Zhang et al[99] introduced miR-206 mimics into human bone marrow-derived mesenchymal stem cell (hBMSC). This miR-206 was then transferred to the human osteosarcoma cell line 143 B via hBMSC-derived exosomes, which subsequently inhibited osteosarcoma growth and spread by targeting transformer 2 protein homolog beta (TRA2B). Zhang et al[57] employed a lentivirus to make adipose-derived mesenchymal stem cell (AD-MSC) express miR-101, using exosomes for targeted delivery, which inhibited the lung metastasis of osteosarcoma. MSC-EVs loaded with miR-22 also inhibited osteosarcoma by targeting the Twist1/CADM1 axis.[100] Thus, engineering MSC-EVs can turn them into excellent carriers for miRNA delivery for the treatment of osteosarcoma.[169,170] However, it remains to be explored whether the other contents of the exosomes may cause a negative effect on the body, and whether miRNAs within exosomes will pair spontaneously reducing efficacy.[171,172] Preparation procedures of exosomes are cumbersome and can only be stored at low temperatures, resulting in high costs, which may limit their widespread use.[173,174] Therefore, more safety validation and clinical trials are needed in order to use engineered MSC exosomes for the clinical treatment of osteosarcoma.[175,176]

Exosomes derived from tumor cells preferentially target tumors because of their homotypic characteristics.[177] In addition, exosomes derived from osteosarcoma cells can serve as miRNA delivery vectors. Shimbo et al[101] introduced miR-143 into human osteosarcoma cell line 143 B. The resulting 143B-derived exosomes then conveyed miR-143 back to 143 B cells, inhibiting their migration. Recently, induction of ferroptosis in tumor cells has become a new direction in tumor therapy.[178,179] Engineering exosomes to induce ferroptosis in tumor cells is a promising therapy strategy of tumor.[180] Jiang et al[102] enhanced the expression of miR-144-3p in the human osteosarcoma cell line, 143 B. miR-144-3p then promoted ferroptosis, curbing osteosarcoma growth and spread by modulating ZEB1 expression. However, exosomes originating from tumors contain elements that can promote cancer progression.[44] Drug resistance of osteosarcoma cells can be transmitted via miRNAs.[181] Therefore, the use of tumor-derived exosomes as miRNA carriers in cancer therapy poses potential risks. Future research should explore the viability of tumor-derived exosomes as carriers for cancer treatment.

MiRNA Delivery via Inorganic-Based Nanoparticles

Magnetofection is an delivery strategy that combines magnetic drug targeting and gene delivery.[182] The kinds of magnetic nanoparticles (MNPs), coating molecules on the surface that can affect their size and charge, external magnetic field, method of administration will influence their biodistribution.[183,184] Surface functionalization of MNPs enhances their ability to adsorb miRNAs and prevents biodegradation.[185] The use of ammonium terminal groups on the nanoparticle surface allows the electrostatic interactions between nanoparticles and miRNAs.[186] So enhancing magnetic nanoparticles with specific substances, such as the cationic polymer PEI, can boost their transfection efficiencies.[183] While iron oxide nanoparticles show significant promise in biomedicine, they have a drawback in terms of their hydrophobic surfaces.[187] The modification of iron oxide with dextran can counteract this limitation.[106] Gong et al[103] used a magnetic gene carrier composed of PEI, dextran, and iron oxide nanoparticles (PDIs) to deliver miR-302b, targeting YOD1 deubiquitinase (YOD1) and suppressing osteosarcoma. In their cytotoxicity tests, the toxicity of PDI rose with increasing concentrations of low molecular weight (≤2000) PEI. However, at low concentrations, its toxicity was minimal, especially when compared to that of PEI with a molecular weight of 25 kDa.[103] Lin et al[188] created

a chitosan-PEI crosslinked polymer (Chi-xPEI) by crosslinking low-molecular-weight PEI with chitosan. This significantly reduced the cytotoxicity of PEI. Additionally, NP-Chi-xPEI, produced by combining iron oxide nanoparticles with Chi-xPEI, demonstrated superior drug-loading capabilities that were reliant on PEI.[188] Thus, modifying PEI cuts down requirement for miRNAs, offers lower cytotoxicity, and enhances the drug-loading efficiency of the inorganic nanoparticles. This approach capitalizes on strengths while mitigating weaknesses. Gong et al[103] highlighted the limitations in drug release and intratumoral distribution of PDI. Studies have suggested that surface functionalization can address these issues.[189,190]

Photothermal therapy (PTT) involves irradiation of a photosensitizer with a near-infrared laser to produce high temperatures that kill cancer cells. This method has been extensively studied for the treatment of bone cancer.[191] Li et al[104] combined miR-520a-3p with polydopamine (PDA)-coated, folate-modified iron oxide (Fe_2O_3@PDA-FA) for osteosarcoma PTT, which effectively inhibited its growth. PDA modification enhances the biocompatibility and hydrophilicity of iron oxide and offers robust photothermal conversion capabilities suitable for PTT.[192] The folate receptor is overexpressed in numerous tumor cells, allowing folate-modified nanocarriers to specifically target them.[193–195] Similarly, Luo et al[196] employed a multifunctional nanoplatform crafted from $FePS_3$ and poly-L-lysine-PEG-folic acid (PPF) (FePS@PPF), to convey anti-miR-19a, suppressing osteosarcoma growth. Li et al[104] utilized an 808 nm near-infrared (NIR) laser for irradiation, whereas Luo et al[196] employed a 1064 nm NIR-II laser. The chosen irradiation wavelength depends on the photothermal conversion efficiency and the depth of lesion.[197] And for osteosarcomas that are deep in the tissue, long wavelength, such as the NIR-II window (1000–1700 nm), is required for effective treatment.[105] Moreover, the control of the duration, temperature level and uniformity of photothermal therapy at the lesion site also affects its clinical translatability.[198] Except from cancer, Fe_2O_3@PDA-FA and FePS@PPF mainly aggregated in the spleen, and their metabolic patterns and effects on the body still needed to be further clarified.[104,196] In conclusion, merging the photothermal therapy with miRNA genes targeting, especially when utilizing inorganic nanocarriers, holds great promise.

Directions and Challenges in the Future

MiRNAs, which are key players in epigenetic regulation, have demonstrated potent regulatory and therapeutic effects on tumors with aberrant gene expression. MiRNAs are promising diagnostic biomarkers of osteosarcoma.[199] And then numerous miRNAs have been linked to the onset and progression of osteosarcoma, positioning them as potential therapeutic targets.[200] By analyzing expression profiles of miRNAs across various osteosarcoma types, specific miRNAs can be identified for targeted treatment.

MiRNAs are susceptible to be cleared by RNase in vivo, so they need to be protected and delivered by vectors.[32,201] Targeted miRNA delivery for tumor therapy is a burgeoning research area with immense potential. Safety remains paramount when selecting delivery vectors. While viral vectors offer robust transfection capabilities, they pose safety concerns.[202] In contrast, nonviral vectors may have limited drug-loading capacity but are generally safer. Current researches on miRNA delivery for osteosarcoma treatment employed nanocarriers, such as polymers, liposomes, exosomes, and inorganic particles. Cationic polymers can be cytotoxic because of their abundant amino groups.[203] Intravenous injection of large liposomes has a risk of causing embolism.[155,156] Exosomes excel in drug loading and biocompatibility; however, some studies have suggested that they may inadvertently promote cancer growth.[164–166] The exact metabolism of inorganic-based nanoparticles in the body still needs to be explored.[104,196] Fortunately, with proper engineering, these challenges associated with nanocarriers can be addressed.

Modifications are crucial for enhancing the nanocarrier performance. While abundant surface amino groups of PEI contribute to its cytotoxicity, modifications, such as ethylenediamine, can mitigate this effect.[139] Using low molecular weight (≤2000) PEI to modify nanocarriers can reduce positive charge cytotoxicity while retaining its advantages.[103] Additionally, suitable modifications can enhance the properties of polymeric supports. For instance, HA and folate modifications can enhance the tumor-targeting ability of nanocarriers.[117,118] PEGylation extends the blood retention time of nanocarriers, thereby facilitating drug release.[109] Choosing engineered exosomes from MSCs to inhibit osteosarcoma may address the issue of safety.[167] These solutions have significant potential for safety.

Given that osteosarcoma tends to metastasize early, the mode of administration is worth being considered. In the review, miRNA delivery modalities used to treat osteosarcoma are categorized into local and systemic applications. Chen et al[96] successfully inhibited osteosarcoma metastasis by local application of miRNAs. However, osteosarcoma tends to metastasize early and often has been metastatic by the time it is diagnosed.[204,205] Zhang et al[57] shrank pulmonary metastasis of osteosarcoma by tail vein injection of miR-101 encapsulated in exosomes from adipose-derived mesenchymal stem cells. Therefore, systemic delivery might be suitable for osteosarcoma, especially for metastasis. More experiments are needed to explore the difference in efficacy between systemic and topical drug administration.

Combining miRNA delivery with other therapies offers promising potential for osteosarcoma treatment. When combined with chemotherapeutic drugs, miRNAs enhance the chemosensitivity of osteosarcoma, significantly improving the efficacy of chemotherapy.[94] The integration of PTT with miRNA delivery holds considerable promise.[104,196]

Several miRNA-based therapies have been tested in clinical trials.[22,206,207] However, no miRNA delivery therapy for osteosarcoma has entered clinical trials. Osteosarcoma development is characterized by intricate molecular mechanisms and alterations in the expression of several miRNAs.[68] Gaining insight into the regulatory networks and associated signaling pathways of miRNAs in osteosarcoma could pave the way for initiating clinical trials of miRNA delivery.

Recently, plant-derived nanoparticles have become a hot research topic.[208] Plant-derived exosome-like nanoparticles are widely available which relatively saves the cost, and are capable of delivering miRNAs.[209] Thus, the feasibility of using plant-derived exosome-like nanoparticles to deliver miRNAs for the treatment of osteosarcoma could be explored in the future.

In summary, when modified with specific substances, the capabilities of nanocarriers can be optimized, making them highly effective for miRNA delivery in osteosarcoma treatment. Compared to exosomes whose contents are uncertain, polymers, liposomes, and inorganic-based nanoparticles have relatively more defined influence on the organism and thus have advantages in clinical applications. Furthermore, integrating miRNA delivery with therapies such as chemotherapy and PTT presents vast potentials, warranting additional researches. In conclusion, multiple kinds of carriers and miRNAs can be combined into a lot of delivery systems, which generates various research orientations in the future.

Conclusion

Overall, miRNA delivery holds significant promise for osteosarcoma treatment, particularly when combined with other therapies. However, the application of nanocarriers for miRNA delivery in osteosarcoma treatment remains in the early stages of development. The in vivo metabolism mechanism, toxicity, mode of administration, and dosage of miRNA delivery vectors still need to be clarified through further studies to accelerate clinical translation. In conclusion, however, miRNA delivery holds great promise for the treatment of osteosarcoma.

Ethics Approval and Consent to Participate

Not applicable. Ethical approval was not required as this umbrella review is a synthesis and analysis of existing studies.

Consent for Publication

All authors declare full consent for publication.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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