Modulation of immunosuppressive cells and noncoding RNAs as immunotherapy in osteosarcoma

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The most common bone cancer is osteosarcoma (OS), which mostly affects children and teenagers. Early surgical resection combined with chemotherapy significantly improves the prognosis of patients with OS. Existing chemotherapies have poor efficacy in individuals with distant metastases or inoperable resection, and these patients may respond better to novel immunotherapies. Immune escape, which is mediated by immunosuppressive cells in the tumour microenvironment (TME), is a major cause of poor OS prognosis and a primary target of immunotherapy. Myeloid-derived suppressor cells, regulatory T cells, and tumour-associated macrophages are the main immunosuppressor cells, which can regulate tumorigenesis and growth on a variety of levels through the interaction in the TME. The proliferation, migration, invasion, and epithelial–mesenchymal transition of OS cells can all be impacted by the expression of non-coding RNAs (ncRNAs), which can also influence how immunosuppressive cells work and support immune suppression in TME. Interferon, checkpoint inhibitors, cancer vaccines, and engineered chimeric antigen receptor (CAR-T) T cells for OS have all been developed using information from studies on the metabolic properties of immunosuppressive cells in TME and ncRNAs in OS cells. This review summarizes the regulatory effect of ncRNAs on OS cells as well as the metabolic heterogeneity of immunosuppressive cells in the context of OS immunotherapies.

KEYWORDS

osteosarcoma, noncoding RNA, immunosuppressive cells, TME, immunotherapy, metabolic heterogeneity

Introduction

Osteosarcoma (OS) is a malignant mesenchymal tumour that most commonly affects children and adolescents and has a high rate of metastasis and mortality (1). OS primarily affects the epiphysis of the long bones in the extremities, with lung metastases occurring most frequently (2). Current treatments for OS include surgical resection and adjuvant chemotherapy, which typically result in a survival rate of less than 5 years for patients with distant metastases (3). Metastatic OS has been successfully treated with immunotherapy, and the mechanisms underlying this success are related to the heterogeneity of immunosuppressive cells in metastatic tumours and the interaction of stromal and immunosuppressive cells in the tumour microenvironment (TME) (4).

The TME in OS is complex and diverse and plays a critical role in tumorigenesis and development. The TME consists of stromal cells and other key factors, including cancer-associated fibroblasts (CAFs), immune cells, extracellular matrix, and vasculature (5). To promote the occurrence and development of tumour cells, stromal cells secrete cytokines, growth factors, and chemokines (6). Immune cells such as lymphocytes and natural killer cells can effectively control tumour invasion, which can be suppressed by immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), and tumour-associated macrophages (TAMs) (7). Immunosuppressive cells and stromal cells in the TME mutually promote the growth and maturation of OS cells (8).

The proliferation, angiogenesis, and apoptosis of OS cells are closely related to noncoding RNAs (ncRNAs), including microRNAs (miRNAs), long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs) (9, 10). miRNAs can regulate the proliferation and apoptosis of OS cells *via* their aberrant expression (11). Overexpression of mir-542-5p can enhance proliferation, but miRNA-1236-3p can decrease proliferation and promote apoptosis in OS cells (12, 13). lncRNAs can enhance OS progression, such as SNHG3, whose overexpression can speed up the migration and invasion of OS cells (14). circRNAs function as a miRNA sponge, regulating transcriptional or post-transcriptional gene expression and contributing to the control of OS incidence and development (15). lncRNA and circRNA can regulate the biological activity of OS cells by forming miRNA sponge, which act as competitive endogenous RNA (ceRNA) (10, 16). Studies on the metabolic properties of immunosuppressive cells and ncRNAs in OS cells promote the use of immunotherapy in the treatment of OS, including interferon treatments, checkpoint inhibitors, cancer vaccines, and engineered chimeric antigen receptor T (CAR-T) cells (4, 17). Among these, CAR-T cell treatment offers a significant advancement in T-cell-based immunotherapy and is predicted to be a game changer in OS immunotherapies (18). We summarize the metabolic properties of immunosuppressive cells in the TME

and functional ncRNAs in OS in this paper. The targets, efficacy, and drug resistance of several recently developed immunotherapies are compared.

Noncoding RNAs in osteosarcoma

The pathophysiology of OS is related to aberrant oncogene activation and tumour suppressor gene inactivation induced by somatic mutations and epigenetic processes (19). Recent studies have increasingly focused on the dysregulation of ncRNAs, including miRNAs, lncRNAs, and circRNAs (9, 20).

MicroRNAs

miRNAs regulate cell proliferation, differentiation, apoptosis, and development by binding to the 3' untranslated region (3'-UTR) of target mRNAs and are able to degrade or induce translational silencing in OS cells (21). miR-223-3p has been shown in studies to limit cadherin-6 expression by directly binding to the 3'-UTR of cadherin-6 and to inhibit the invasion, migration, growth, and proliferation of OS cells (22). The expression of miR-18b-5p, which is mediated by HIF-1α, is substantially increased in OS and is associated with a poor prognosis (23). In addition, miR-18b-5p promotes the incidence and development of OS by inhibiting the expression of the tumour suppressor gene PHF2 (23). miRNA-98-5p is under-expressed in OS and inhibits cell cycle progression and migration potential by down-regulating CDC25A, thereby inducing OS apoptosis (24). Overexpression of miRNA-1236-3p in HOS cells reduces proliferation, stops the cell cycle in the G0/G1 phase, and promotes apoptosis (13). A differential analysis of miRNA expression in OS (Figure 1A) shows that the expression of let-7A-2 and miR-323 is decreased, whereas the expression of miR-182 is increased, suggesting that miR-182 could be a possible therapeutic target in OS. The detailed information of differentially expressed ncRNAs in A-C is presented in Table S1.

Long noncoding RNAs

The expression of lncRNA MELTF-AS1 is significantly increased in OS and promotes OS metastasis by upregulating the expression of MMP14 (25). lncRNA ODRUL can act as a competitive endogenous RNA (ceRNA) sponge of miR-3182 and promotes the proliferation, migration, invasion, and tumour growth of OS by upregulating the expression of matrix metalloproteinase (MMP) II (26). The oncogenic
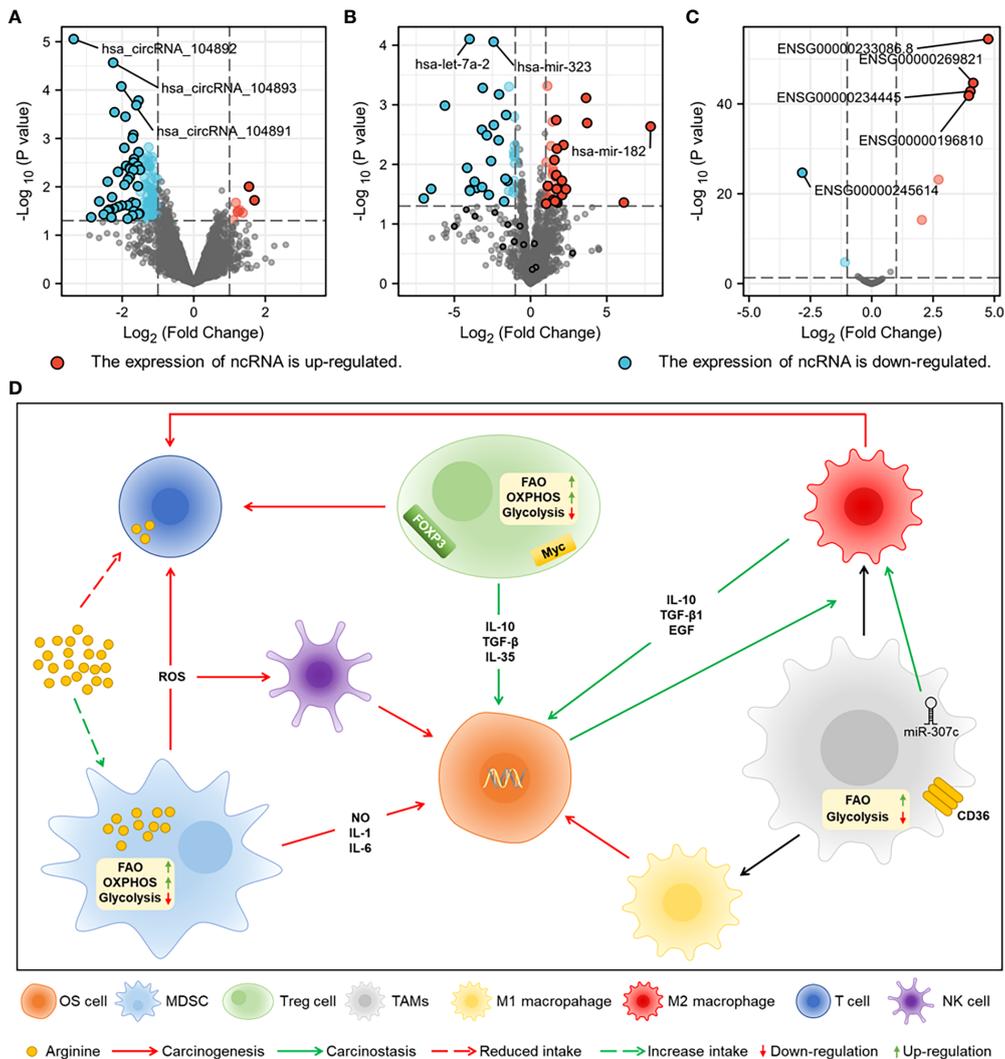
FIGURE 1

The sequencing results of ncRNAs from normal tissues and OS tissues of human are downloaded from GEO datasets of National Center for Biotechnology Information (NCBI) for difference analysis. **(A)** Differential expression analysis of miRNAs (GSE70367). **(B)** Differential expression analysis of lncRNAs (GES156344). **(C)** Differential expression analysis of circRNAs (GSE96964). **(D)** Metabolic characteristics of MDSC, TAMs and Treg cells in TME and crosstalk of them with NK cells, T cells and OS cells. MDSC promotes the growth of OS cells by secreting inflammatory factors, damages T cells and NK cells by secreting ROS, and competitively consumes arginine with T cells. Treg cells inhibit T cell function by expressing FOXP3 and myc. CD36 receptor and miR-307c in TAMs promote M2 polarization, and FAO is the main energy metabolism.

effects of LncRNA CBR3-AS1 are executed by regulating the network of the miR-140-5p/DDX54-NucKS1-mTOR signalling pathway, which encourages stemness and epithelial–mesenchymal transition (EMT) of OS (27). The overexpression of lncRNA EBLN3P promotes the progression of OS cells, which is indicative of the stimulating effects of EBLN3P (28). In OS cells, the expression of the lncRNAs ENSG00000233086.8 and ENSG00000269821 is much higher (Figure 1B). By examining the molecular pathways and regulatory mechanisms further, one may be able to control the development of OS.

Circular RNAs

circECE1 is highly expressed in OS tissues and cells, and its association with c-Myc promotes tumour proliferation and metastasis by boosting glucose metabolism in OS cells to prevent speckle-type POZ-mediated ubiquitination and degradation of c-Myc (29). C-Myc-targeting checkpoint inhibitors have been demonstrated to impede OS development *via* modulating the production of ncRNAs (30). Studies have shown that knockdown of circRNA circ_001422 significantly inhibits the proliferation and metastasis of OS cells and

promotes apoptosis. Regulating the miR-195-5p/FGF2/PI3K/ AKT axis produces the opposite impact of overexpression (31). circMYO10 has been confirmed as a promoter of OS progression by regulating the miR-370-3p/RUVBL1 axis and chromatin remodelling, consequently boosting the transcriptional activity of the β-catenin/LEF1 complex (32). The number of circRNAs with decreased expression was much greater than those with enhanced expression (Figure 1C), a finding that could be leveraged to design targeted therapies once the regulatory mechanisms of these circRNAs have been elucidated.

Recent research has increasingly focused on the impacts and mechanisms of microRNAs, whereas research into lncRNAs and circRNAs is still in its infancy (Table 1) (102). More research points to the importance of noncoding RNAs in OS, both in terms of diagnosis and treatment (9). An alternative mechanism for OS chemotherapeutic resistance has been proposed through the construction of ceRNA networks, in which noncoding RNAs bind to mRNAs (103). Differential expression of noncoding RNAs and the formation of ceRNA networks may lead to the development of more effective treatment techniques and the ability to overcome drug resistance in OS (Figure S1).

Immunosuppressive cells in osteosarcoma

Myeloid-derived suppressor cells

Immature bone marrow cells (IMCs) differentiate into mature macrophages, dendritic cells, and granulocytes under physiological conditions and transform into immunosuppressive MDSCs when regulated by chemokines in the TME (104). MDSCs generate pro-inflammatory substances such as NO, IL-1, and IL-6, which expose OS cells to a persistently inflammatory environment and dramatically enhance the risk of DNA damage and tumour cell proliferation, which may contribute to the progression of OS (105, 106). Through the activation of the activator for transcription 3, miR-21 and IL-6 can synergistically enhance the development of MDSCs and influence treatment resistance (107). Reactive oxygen species (ROS) produced by oxidative stress can activate the NF-κB and Nrf2 pathways, allowing tumour cells to survive (108). MDSCs generate excessive ROS via NOX2 and suppress the antitumor effects of T cells and natural killer (NK) cells, hence mediating OS immune escape while maintaining oxidative balance via glycolysis upregulation (109, 110).

The TME alters the lipid metabolism of MDSCs to enhance the uptake of fatty acids and the activation of fatty acid oxidation (FAO), thereby improving the immunosuppressive activity of MDSCs and promoting tumour growth (110). In addition to LXR agonists, liver-X nuclear receptors (LXRs) regulate cholesterol and lipid metabolism via the transcription target Apolipoprotein E (111). LXR agonists have been demonstrated to play a role in MDSC depletion, which could be related to FAO inhibition in MDSCs (112). By increasing the activities of arginase-1, MDSCs compete with T cells for the consumption of arginine, which leads to T cell dysfunction (113). L-arginine supplementation may improve the anticancer impact of cyclophosphamide (CP) and minimize T cell dysfunction caused by increased MDSCs generated by CP (114).

Tumour-associated macrophages

TAMs are the primary immune cells in the TME, which are usually produced from bone marrow monocytes, and the presence of TAMs is indicative of a poor prognosis in OS patients (115, 116). TAMs, via stimulating the COX-2/STAT3 axis and causing epithelial– mesenchymal transition, can increase OS pulmonary metastasis (117). C–C motif chemokine ligand 18 secreted by TAMs has been shown to promote the proliferation and metastasis of OS cells via the EP300/UCA1/Wnt/β-catenin pathway, which significantly reduces the survival rate of OS patients (118). Studies have demonstrated that miR-363 inhibitors can promote the migration of TAMs after transfection of OS cells (119).

TAMs can be divided into classically activated macrophages (M1), with antitumor activity, and selectively activated macrophages (M2), with tumour-promoting activity, both of which can coexist in the TME (120). It has been found that M2 can promote the deterioration of OS cells through the SOCS3/JAK2/STAT3 axis, and OS cells can enhance the M2 polarisation of TAMs (121). LncRNA RP11-361F15.2 enhances M2 polarisation mediated by cytoplasmic polyadenylate element binding protein 4 through miR-30c-5p and further promotes the occurrence of OS (122).

TAMs substitute glycolysis with FAO as a source of energy by expressing a high amount of the scavenger receptor CD36, which enhances lipid accumulation and reprograms TAMs into M2 types (123). S100A4 has been reported in mice to upregulate FAO and mediate TAM polarization to M2, as well as to have carcinogenic activity (124).

Treg cells

Extensive Treg cell infiltration into tumour tissues is often associated with a poor prognosis, whereas their removal enhances antitumor immune responses (125). FOXP3+ expression in Treg cells has been shown to predict the prognosis of osteosarcoma in vivo and in vitro and could potentially be used as a diagnostic marker in clinical practice (126–128).

TABLE 1 Regulation of miRNAs, lncRNAs and circRNAs in OS cells.

Non-coding RNA	Expression	Function	Ref
miR-873	upregulate	Related to tumor size, clinical stage and distant metastasis in OS.	(33)
miR-23b-3p	upregulate	Inhibit OS cell proliferation.	(34)
miR-367	upregulate	Inhibit the proliferation, migration and invasion of OS cells.	(35)
miR-21	upregulate	Play a main role in proliferation, migration, invasion and apoptosis.	(36)
miR-107	upregulate	Promoted U_2OS cell viability, migration, and invasion whereas inhibit apoptosis.	(37)
miR-590-3p	downregulate	Inhibit proliferation and metastasis in OS cells.	(38)
miR-520a-3p	downregulate	Tumor suppressor.	(39)
miR-491	downregulate	Stimulate OS cell lung metastasis and suppresses CDDP-induced tumor growth inhibition and apoptosis.	(40)
miR-449a	downregulate	Decrease cyclin A2 levels and inhibit proliferation rate, migratory potential, and colony-forming ability of OS cells.	(41)
miR-432-5p	downregulate	Regulate SA and IA by targeting PDGFB genes.	(42)
miR-425-5p	downregulate	Suppress OS cell proliferation, invasion and migration *in vitro*.	(43)
miR-424	downregulate	Decrease cyclin A2 levels and inhibited proliferation rate, migratory potential, and colony-forming ability of OS cells.	(41)
miR-377	downregulate	Inhibit tumor growth and reduce tumor size.	(44)
miR-363-3p	downregulate	Inhibit the proliferation, migration, and invasion of U_2OS and MG63 cells.	(45)
miR-342-3p	downregulate	Inhibit the proliferation, migration, and invasion of OS cells.	(46)
miR-26a	downregulate	Suppress the malignant behaviors of OS cells.	(47)
miR-223-3p	downregulate	Inhibit cell invasion, migration, growth, and proliferation.	(22)
miR211	downregulate	Increase the percentage of cell apoptosis, and suppress cell proliferation as well as cell migration/invasion.	(48)
miR-133b	downregulate	Attenuate cell proliferation and invasion.	(49)
miR-520b	downregulate	Inhibit cell proliferation, migration, and invasion.	(50)
miR-326	downregulate	Promote the proliferation and invasion of MG63 cells as well as the growth and metastasis in nude mice.	(51)
lncRNA MALAT1	upregulate	Promote OS cell viability, invasion and migration.	(52)
lncRNA TP73-AS1	upregulate	Suppress OS cells proliferation and invasion *in vitro* as well as tumor growth *in vivo*.	(53)
lncRNA HNF1A-AS1	upregulate	Inhibit cell proliferation and G1 /S transition, and suppress migration and invasion in OS cells.	(54)
lncRNA-BC050642	upregulate	Promote cell proliferation, induce colony formation and meanwhile inhibit cell apoptosis.	(55)
lncRNA ODRUL	upregulate	Inhibit OS cell proliferation, migration, invasion, and tumor growth *in vitro* and *vivo*.	(26)
lncRNA ITGB2-AS1	upregulate	Inhibit the proliferation and induce apoptosis of OS cells.	(56)
lncRNA ANRIL	upregulate	Associate with increased rates of metastases at diagnosis and death. A significant predictor of reduced overall survival rate.	(57)
lncRNA XIST	upregulate	Responsible for OS cell proliferation and invasion.	(58)
lncRNA TUG1	upregulate	Play an important role in the proliferation and metastasis of osteosarcoma.	(59)
lncRNA TUG1	upregulate	Regulate OS cell metastasis, angiogenesis, and proliferation *in vivo* and *vitro*.	(60)
lncRNA TNK2-AS1	upregulate	Inhibited proliferative, migratory, and invasive capacities while promoting apoptosis in OS cells.	(61)
lncRNA SNHG4	upregulate	Suppress cell viability and invasive potential.	(62)
lncRNA SNHG3	upregulate	Promote invasive and migratory potentials of OS cells.	(14)
lncRNA SNHG1	upregulate	Inhibit cell growth and metastasis of OS *in vitro* and *vivo*.	(63)
lncRNA SNHG16	upregulate	Contributes to the proliferation, migration and invasion of OS cells.	(64)
lncRNA OIP5-AS1	upregulate	Increased doxorubicin resistance of OS cells.	(65)
lncRNA OIP5-AS1	upregulate	Repress the proliferative ability and accelerated the apoptosis.	(66)
lncRNA MIR100HG	upregulate	Suppress cell proliferation, cell cycle progression while promote cell apoptosis.	(67)
lncRNA LINC01123	upregulate	Promote cell progression.	(68)
lncRNA LINC00324	upregulate	Accelerate the proliferation and migration of OS cells.	(69)
lncRNA KCNQ1OT1	upregulate	Facilitate proliferation and suppressed apoptosis of OS cells.	(70)
lncRNA JPX	upregulate	Elevate the cell viability and proliferation.	(71)
lncRNA HULC	upregulate	Promote OS cell proliferation, migration and invasion and induce cell apoptosis.	(72)

(Continued)

TABLE 1 Continued

Non-coding RNA	Expression	Function	Ref
lncRNA HOXD-AS1	upregulate	Suppress cell proliferation, colony formation, migration, and invasion, and promote cell cycle arrest at G1 stage and apoptosis in OS cells.	(73)
lncRNA HOXD-AS1	upregulate	Inhibit the OS cells proliferation and induce G1/G0 phase arrest *in vitro*, and repress tumor cell growth *in vivo*.	(74)
lncRNA FOXD2-AS1	upregulate	Repress the malignant biological properties of OS cells *in vitro* and *vivo*, including proliferation, invasion, apoptosis and tumor growth.	(75)
lncRNA DLEU1	upregulate	Inhibit the cell proliferation, migration and invasion.	(76)
lncRNA DANCR	upregulate	Promote tumor growth and lung metastasis of OS.	(77)
lncRNA DANCR	upregulate	Increase OS cell proliferation, migration, and invasion.	(78)
lncRNA CCAT2	upregulate	Promote OS cell proliferation, cell cycle and invasion.	(79)
lncRNA CBR3-AS1	upregulate	Suppress OS cells proliferation, migration and invasion, and promote cells apoptosis.	(80)
lncRNA APTR	upregulate	Repress human OS cell proliferation, invasion and migration, and induce apoptosis.	(81)
lncRNA CAT104	upregulate	Inhibit OS-732 cell proliferation, migration, and invasion, but promote cell apoptosis.	(82)
lncRNA LINC01128	upregulate	Reduce the proliferation, migration and invasion of OS cells both.	(83)
lncRNA ZBTB7A	upregulate	Associate with OS metastasis.	(84)
lncRNA RSF1	upregulate	Suppress OS cells proliferation and invasion.	(85)
lncRNA PUM2	downregulate	Inhibit OS cells proliferation, migration, and stemness.	(86)
lncRNA XIST	downregulate	Inhibit the proliferation of OS cells.	(87)
lncRNA C2dat1	downregulate	Reduce cell viability, invasion, and migration, whereas increase cell apoptosis in OS-732 cells.	(88)
hsa_circ_0008934	upregulate	Reduce proliferation, enhanced apoptosis, block cell cycle progression, and impair migration and invasion capacities of SAOS$_2$ cells.	(89)
hsa_circ_0007534	upregulate	Suppress OS cell growth.	(90)
circUSP34	upregulate	Promote the proliferation, migration, and invasion of OS *in vitro* and *vivo*.	(91)
circ-LRP6	upregulate	Inhibit the proliferation, migration and invasion of OS cells.	(92)
circUBAP2	upregulate	Inhibit OS cell apoptosis.	(93)
circTADA2A	upregulate	Increase malignant tumor behavior.	(94)
hsa_circ_0002137	upregulate	Suppress the progress of OS, including cell invasion, cell cycle and cell apoptosis.	(95)
circPVT1	upregulate	Suppress OS tumor growth and metastasis *in vivo*.	(96)
circECE1	upregulate	Suppress tumor proliferation and metastasis both *in vitro* and *vivo*.	(29)
circ_0078767	upregulate	Strengthen the proliferation, invasiveness, and migration of osteosarcoma cells.	(97)
circ_001621	upregulate	Promote OS proliferation and migration.	(98)
circ_001422	upregulate	Promote the proliferation and metastasis and inhibit the apoptosis of OS cells *in vivo* and *vitro*.	(31)
circ_0001721	upregulate	Facilitates cell progression in OS.	(99)
circ-0000658	downregulate	Promote cell cycle, proliferation, invasion and migration but inhibit the apoptosis of OS cells.	(100)
circ-0000190	downregulate	Exhibit an obvious reduction in tissues of OS patients.	(101)

Glycolysis and oxidative phosphorylation, which are essential for Treg cell metabolism, require FAO (129). Treg cells in tumours, in contrast to normal tissues, have considerably decreased glucose uptake and are dysfunctional in a high-glucose environment (130). P13K inhibitors can reduce the immunosuppressive effects of Treg cells by upregulating glycolysis and reducing FOPX3 expression (131). miR-34a targets the 3' UTR to inhibit the expression of FOXP3, which is controlled by the NF-κB pathway and downregulated by IL-6 and TNF-α (132). It has been demonstrated that the transcriptional regulator c-Myc influences oxidative phosphorylation in Tregs *via* regulating mitochondrial activity, hence limiting accumulation and functional activation (133). Targeting c-Myc and associated signalling pathways as a means of treating OS has drawn a lot of interest (29, 134).

Immunosuppressive cells can regulate the occurrence and development of OS through crosstalk with stromal cells in the TME (Figure 1D), which are regulated by ncRNAs in OS cells, according to the studies on the metabolic heterogeneity of immunosuppressive cells and the regulatory mechanisms of ncRNAs.

Immunotherapy in osteosarcoma

Interferon therapy

Interferon (IFN) is a cytokine that white blood cells generally secrete during infections (135). Due to its effects as an agonist of antitumor activity in adaptive and innate immune cells, it leads to the establishment of antiproliferative and antiangiogenic activity in osteosarcoma and antagonizes inhibitory immune subsets (135, 136). IFN-γ induces PKR-dependent autophagy in OS cells through signal transduction and activation of transcription 1, phosphatidylinositol 3-kinase, and mitogen-activated protein kinase-dependent pathways (137). miR-142-5p enhances the transcription of IFN-γ by downregulating the expression of interaction protein domain 2 (138). miR-31 reduces interferon-γ production, thereby attenuating Th1 response (139). The efficiency of IFN therapy could be increased by modulating the aberrant expression of ncRNAs, which has a good synergy for drug development in the treatment of OS.

Checkpoint inhibitors

PD-1

In the tumour microenvironment of OS, PD-L1 on tumour cells interacts with PD-1 on T cells to inhibit T cell functional signalling, preventing the immune system from targeting tumour cells (140, 141). The antitumor activity of PD-1 can be aided by an SGLT2 inhibitor, and the synergistic effect stimulates the infiltration of CD4^{+} and CD8^{+}T lymphocytes into the OS tumour microenvironment (142). miR-140 was found to directly regulate the expression of PD-L1 by binding to its 3'-UTR, suggesting that it could be exploited as a new therapeutic drug targeting checkpoint inhibitors in OS (143). PBMC-loaded vMyx-hTNF may synergistically interact with the immune checkpoint inhibitor anti-PD-1, which has been reported in a mouse model of lung metastatic osteosarcoma (144).

C-Myc inhibitors

The ubiquitous dysregulation of the c-Myc oncogene in human malignancies makes it a promising therapeutic target (145). Recent research has demonstrated that c-Myc not only regulates cell proliferation, apoptosis, and cancer metabolism, but also the TME and immune responses (145). C-Myc inhibition reprograms the cancer immune milieu by attracting T lymphocytes and activating the CD40/CD40L system in OS, according to studies (30). miR-449c has been demonstrated to directly target and negatively inhibit the production of the oncogene c-Myc, hence encouraging the advancement of the OS cell cycle (146). Her4 can boost glucose intake and tumour growth by promoting OS metabolic reprogramming *via* a c-Myc-dependent signalling

pathway, suggesting that a c-Myc inhibitor may be useful in the treatment of OS (147). The S1P/S1PR3 axis has been shown to contribute to the formation of the YAP–c-Myc complex and transcription of the glycolytic enzyme PGAM1 by suppressing YAP phosphorylation and increasing its nuclear translocation, according to studies (134).

SGLT2 inhibitors

Sodium–glucose cotransporter 2 (SGLT2) is essential for epithelial glucose transport and is overexpressed in numerous cancer types in order to supply cancer cells with glucose to satisfy their high-energy needs (148). SGLT2 affects the expression of miR-210 and stimulates anaerobic glycolysis, hence modulating the energy metabolism of cancer cells (149). SGLT2 inhibitors significantly inhibit osteosarcoma tumour growth and induce immune cell infiltration *in vivo* by upregulating STING expression and activating the IRF3/IFN-β pathway, which could be attributable to the inhibition of AKT phosphorylation (141).

Cancer vaccines

The protein EWS-FLI1, which is overexpressed in OS, has become a specific Treg antigen for vaccine development (150). EWS-FLI1 inhibits effector T cell responses and has been found circulating in or infiltrating tumours in Ewing patients, resulting in unfavourable clinical outcomes (150). Double sialic ganglioside (GD2) is extensively expressed in osteosarcoma (OS) and soft tissue sarcomas, and immunotherapies including GD2 vaccines have been utilized to treat solid tumor (151). miR-34a can target GD-2 to enhance tumour apoptosis, which is anticipated to be a novel OS target (152). Previous studies developed fusion cell vaccines by chemically fusing human γδT cells with SAOS-2 cells, eliciting cytotoxic T lymphocyte responses against two human OS cell lines that were specific to cancer antigens (153). CD103^{+}cDC1 vaccines produced *in vitro* elicited systemic and long-lasting tumour-specific T cell-mediated cytotoxicity, thereby inhibiting the growth of primary and metastatic osteosarcoma (154).

Engineered chimeric antigen receptor T cells

Chimeric antigen receptor T cell therapy has been shown to be effective in leukaemia and lymphoma, and current studies have increasingly focused on CAR-T therapy for solid tumours, such as OS (155). The efficacy of B7-H3-CAR-T cell therapy in treating solid tumours was initially proven in a model of childhood cancer (156). Following that, the efficacy of B7-H3-CAR-T cells in OS and preventing lung metastasis progression was demonstrated in a dose-

dependent manner in a mouse model with orthotopic OS of the tibia and lung metastases (157). Human EpHA2-directed CAR-T cells can target human OS cells *in vitro*, and the injection of CAR-T cells can eradicate tumour deposits in the liver and lungs of metastatic OS models *in vivo* (158). CD166 is selectively expressed in OS cells and can be used as a new target for CAR-T cell therapy, which has been demonstrated in mice models of OS by injection of CD166.BBζ CAR-T cells (159). Human epidermal growth factor receptor 2 (HER2)-CAR-T cells have entered phase II clinical trials, and the safety and efficacy of this therapy have been demonstrated in a study of 19 patients with HER2-positive solid tumours (160).

To treat OS, immunotherapy particularly targets immune cells and immunosuppressive cells in the TME. ncRNAs play a crucial regulatory role and have the potential to be exploited as synergistic agents for checkpoint inhibitors as well as novel targets for interferon treatments and cancer vaccines. The evidence of clinical data in interferon therapy and checkpoint inhibitors is shown in Table S2. CAR-T cells are a new therapeutic for solid tumours that can eradicate tumour cells from primary and metastatic lesions and may provide a unique immunotherapy treatment for patients with metastatic OS.

Discussion

The most frequent primary malignant tumour in children and adolescents is OS, which has a high rate of metastasis and a poor prognosis (161). A difference in the reduction in expression of let-7a-2 and miR-323 was identified in the differential analysis of ncRNAs in OS cells. let-7a-2 and miR-323 are regarded as sensitive prognostic indicators in a number of malignancies and may have a significant role in the clinical diagnosis of OS (162–164). The expression of circRNAs in OS is mainly decreased, of which circRNA_104892, circRNA_104893, and circRNA_104891 show significant differences in the degree of reduction. Reduced expression of circRNAs often inhibits osteosarcoma migration and invasion and promotes apoptosis, which could be combined with therapeutic targets for OS (165). lncRNA SNHG16 can function as ceRNA of miR-1285-3p to reduce the expression of miRNA, thus promote the proliferation, invasion and migration of OS cells (166). lncRNA regulates the progression of osteosarcoma through the miRNA axis, and there is no evidence for the direct regulation of lncRNA expression (167–170). lncRNA and circRNA can regulate the biological characteristics and metabolism reprogramming of OS by sponging miRNAs to represent as ceRNA (70, 94). The construction of co-expression networks of ncRNAs would be beneficial for studying OS aetiology.

OS immunotherapy primarily targets immunosuppressive cells in the TME, which are regulated by cytokines, chemokines, and an anaerobic environment (171). Gemcitabine effectively inhibited the progression of osteosarcoma by inducing cell apoptosis and inhibiting the accumulation of MDSCs (172).

Additionally, when it binds to specific inhibitors of indoleamine 2, 3-dioxygenase, it can more effectively prevent the recruitment of MDSCs and the differentiation of Treg cells (172). The necessity to find novel targets has led to an increase in the number of studies on regulatory factors in OS cells. Meanwhile, when compared to a single inhibitor, a combination of inhibitors can greatly boost therapeutic efficacy. The energy uptake of immunosuppressive cells is more dependent on FAO and is also regulated by glucose levels in the TME (123, 130).

The development of combination chemotherapy has significantly increased the OS survival rate; however, the evolution of drug resistance has become a critical issue that must be addressed (173). Immunotherapy is a new strategy in the treatment of OS that targets immune cells to activate the immune system and relies on autoimmune responses to fight tumour tissues, an approach that may also be useful in combatting drug resistance (4, 173). Multiple types of checkpoint inhibitors have shown significant anticancer efficacy. The synergistic effects of checkpoint inhibitors and their combination with chemotherapy are promising options for combating drug resistance (4). Therapies based on OS-related antibodies have shown promise when combined with checkpoint inhibitors (154). In recent years, CAR-T cell treatment for OS has demonstrated encouraging results. (HER2)-CAR-T cells have entered phase II clinical trials and are expected to advance the treatment of OS (160).

In conclusion, this review summarizes the role of ncRNAs in OS cells, including their differential expression, as well as the metabolic heterogeneity of immunosuppressive cells in the TME. Emerging immunotherapies have been studied and compared in recent years, and their roles in the clinical diagnosis and treatment of OS have been investigated.

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YX and DoW wrote the manuscript. YP, MC, DuW, ZJ and BL collected the references and prepared figures. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.1025532/full#supplementary-material

SUPPLEMENTARY FIGURE 1
The ceRNA network in OS cells.

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