Abstract. – OBJECTIVE: Osteosarcoma is a common bone sarcoma that often occurs in childhood and adolescence. In recent years, the efficacy of osteosarcoma treatments has been improved by adjuvant chemotherapies and surgical approaches. However, poor prognosis often occurs among osteosarcoma patients due to recurrence, metastasis, or drug resistance problems. Cancer stem cells (CSCs), a specific type of tumor malignant cells with stem cell-like properties, have been reported to be responsible for tumor origination, aggression, metastasis, recurrence, and drug resistance. CSCs have been identified in osteosarcomas treatment, which exhibits self-renewal, multi-potency, and enhanced drug resistance. Therefore, in the present narrative review, we intend to summarize the role of lncRNAs in regulating CSCs and their effectiveness in the treatment of osteosarcoma.

MATERIALS AND METHODS: The databases PubMed (Medline), Web of Science, Embase, Scopus, and Cochrane Library, were used for the presented study. The keywords we used to complete our search are ‘lncRNA’, ‘Stem cell’, and ‘osteosarcoma’. A total of over 800 relevant articles, with a time limit from 2010 to 2021, were identified according to search strategy. Duplicate records and review articles were excluded by their titles and abstracts. Finally, we found about 80 articles matching our inclusion criteria, which included about 13 relevant studies focusing on both the mechanism and effectiveness of osteosarcomas treatment among osteosarcoma patients.

RESULTS: CD133, CD117, ALDH, and Stro-1 are validated as the stem cell biomarkers in osteosarcoma CSCs. Accumulating evidence has revealed that lncRNAs, containing HIF2PUT, SOX2-OT, MALAT1, THOR, B4GALT1-AS1, H19, PVT1, FER1L4, LINK-A, DANCN, and DLX6-AS1, play a potential role in regulating CSCs in osteosarcoma. The drug resistance, inhibition of the relapse, and metastasis in osteosarcoma could be avoided via regulating lncRNAs of targeting CSCs.

CONCLUSIONS: Multiple lncRNAs regulate CSCs in osteosarcoma via various molecular mechanisms. This review demonstrated that the method of eliminating CSCs by targeting these lncRNAs is a safe, effective, and a well-tolerated way for osteosarcoma patients, which shows a broad research prospect in tumor diagnoses and therapies. However, this method should be further demonstrated by better animal models and more clinical experiments.

Key Words: Cancer, Stem cell, Osteosarcoma, Targets, Non-coding RNAs, LncRNAs.

Abbreviations
ALDH 1: Aldehyde dehydrogenase 1; BMSC-EVs: Bone marrow-derived mesenchymal stem cells-derived extracellular vesicles; CeRNAs: Competitive endogenous RNAs; CSC: Cancer stem cell; DANCN: Differentiation antagonizing non-protein coding RNA; EMT: Epithelial-mesenchymal transition; ERβ: Estrogen receptor β; FER1L4: Fer-1-like protein 4; HIF-2α: Hypoxia-inducible factor-2α; HIF2PUT: HIF-2α promoter up-stream transcript; LFS: Li-Fraumeni syndrome; iPSC: Induced pluripotent stem cell; LncRNA: Long non-coding RNA; LINK-A: Long intergenic non-coding RNA for kinase activation; MALAT1: Metastasis-associated lung adenocarcinoma transcript 1; NPC: Nasopharyngeal cancer; PIM: Proviral integration site for Moloney murine leukemia virus; PTC: Papillary thyroid carcinoma; PVT1: Plasmacytoma variant translocation gene 1; SOX-2: SRY-related HMG-box 2; SOX2-OT: SOX2 overlapping transcript; SOCS5: Suppressor of cytokine signaling 5.
Introduction

Osteosarcoma, one of the most common bone malignancies, often occurs among juvenile or teenage patients. With technological development, therapeutic strategies, including adjuvant chemotherapy and surgical approaches, have been further improved in recent years. However, a poor prognosis, such as recurrence and metastasis, often occurs among osteosarcoma patients. To improve the survival rate of osteosarcoma patients, it is necessary to study the mechanism of osteosarcoma development and progression both in-depth and systematically.

CSCs are a specific type of tumor malignant cells, which have cell-like features, which might be responsible for several cellular processes, such as tumor origination, aggression, metastasis, recurrence, and drug resistance. Evidence has shown that CSCs exist in osteosarcomas, which are stem-like cells with unique capacities, such as self-renewal, multipotency, and enhanced drug resistance. Moreover, several stem cell markers of osteosarcoma CSCs have been identified, including CD133, CD117, ALDH, and Stro-1. Strikingly, targeting CSCs could overcome the drug resistance issue and inhibit the relapse and metastasis of osteosarcoma.

LncRNAs in Overall Survival

Long non-coding RNAs (LncRNAs) belong to a family of non-coding RNA with a length higher than 200 nucleotides, which can be transcribed by RNA polymerase II. So far, more than thousands of LncRNAs have been found which can be divided into sense, antisense, intronic, intergenic, bidirectional LncRNAs, according to location sites on the protein-coding genes. LncRNAs perform their functions via serving as miRNA precursor, ceRNAs, and interacting with transcription preinitiation complex within the promoter. LncRNAs have been demonstrated as potentially important factors during the development and progression process of osteosarcoma. One research group from Xiangya Hospital has reported that several LncRNAs could be potential biomarkers for osteosarcoma prediction and treatment targets for osteosarcoma patients. Notably, LncRNAs affect cisplatin resistance in osteosarcoma. In the following sections, we will describe the role of LncRNAs in regulating CSCs in osteosarcoma and their underlying molecular mechanisms.

Targeting CSCs by LncRNAs in Osteosarcoma

Recently, accumulating pieces of evidence have been reported that LncRNAs have a significant effect in regulating CSCs in osteosarcoma and other human cancers. Several studies have been reported, indicating that LncRNAs are involved in the regulation of osteosarcoma CSCs via various mechanisms, including LncRNAs HIF2PUT, SOX2-OT, MALAT1, THOR, B4GALT1-ASI, FER1L4, LINK-A, DANC1, and DLX6-ASI. Thus, the functions and mechanisms of these LncRNAs in osteosarcoma cells are discussed in the following sections.

The Functions of LncRNA HIF2PUT in Osteosarcoma Cells

It is demonstrated that upregulation of HIF-2PUT could suppress proliferation and migration of MG63 cells, while downregulation exhibit the opposite effect. Ectopic expression of HIF2PUT reduced the number of CD133-positive MG63 cells, whereas depletion of HIF2PUT increased the number of those cells. Furthermore, overexpression of HIF2PUT attenuated the sphere-forming capacity of MG63 cells, while HIF2PUT siRNA treatment exhibited the opposite impact. Theoretically, HIF2PUT exerted its biological functions via the promotion of HIF-2a expression in osteosarcoma cells. Hereby, HIF2PUT expression was positively associated with HIF-2a levels in osteosarcoma specimens. Zhao et al revealed that HIF2PUT suppressed proliferation, migration, and invasion process, and also, controlled impaired sphere formation of U2OS and MG-63 via modulating HIF2 expression. This study suggested that LncRNA HIF2PUT could partially regulate the osteosarcoma stem cells via targeting HIF-2a expression. Similar findings of HIF2PUT-mediated suppression of colorectal CSC properties were observed in colorectal cancer. HIF2PUT knockdown by siRNA transfection reduced the expression of stemness genes, repressed spheroid formation capacity via blocking HIF2a expression in DLD-1 and HT 29 CRC cells. Consistently, osteosarcoma patients with higher expression of HIF2PUT and HIF-2a exhibited an unfavorable prognosis. HIF2PUT overexpression was correlated with tumor size, stage, distant metastasis, and overall survival (OS) and disease-free survival (DFS) in osteosarcoma patients. However, one group reported that HIF2PUT expression was upregulated in osteosarcoma, indicating that HIF2PUT might be
an oncogene. Thus, further investigation must be performed to determine the role of HIF2PUT in osteosarcoma generation and progression.

**The Functions of LncRNA SOX2-OT in Osteosarcoma Cells**

LncRNA SOX2-OT has been reported to be an effective LncRNA for osteosarcoma treatment according to several types of tumor tissues. A higher expression level of SOX2-OT was associated with poor survival in cancer patients. LncRNA SOX2-OT was highly expressed in osteosarcoma specimens and several cell lines, such as SaOS-2, MG-63, U2OS, MNNG/HOS cell lines. Moreover, LncRNA SOX2-OT expression was related to clinico-pathological characteristics in patients with osteosarcoma, including tumor size, stage, histological grade, distant metastasis, and overall survival. Overexpression of LncRNA SOX2-OT stimulated proliferation and motility of SaOS-2 osteosarcoma cells, while depletion of LncRNA SOX2-OT exhibited the opposite effects in U2OS cells. Moreover, LncRNA SOX2-OT positively regulated the expression of SOX2 in osteosarcoma cells. Furthermore, LncRNA SOX2-OT constrained the expression of multiple CSC biomarkers, such as CD44, CD133, OCT4, ALDH1, and NANO. This study indicated the potential effect of SOX2-OT in regulating osteosarcoma CSCs via targeting SOX2.

**The Functions of LncRNA MALAT1 in Osteosarcoma Cells**

LncRNA MALAT1 has been observed to be overexpressed in osteosarcoma patients. MALAT1 enhanced tumor metastasis and cell proliferation via serving as a ceRNA of miR-144-3p and inhibited ROCK1/ROCK2 expression in osteosarcoma cells. MALAT1 overexpression was correlated with tumor invasion, metastasis, and prognosis in osteosarcoma patients. Ectopic expression of MALAT1 increased proliferative activity and enhanced migratory and invasive capacity of osteosarcoma cells, as well as promoted tumorigenicity for mice, while MALAT1 inhibition exerted the opposite functions. MALAT1 overexpression upregulated the expression of several stemness biomarkers, such as CD90, CD133, and SOX2, whereas the expression of these stemness markers was decreased after MALAT1 down-regulation in SW1353 and SOSP-9607 cells. The proportion of CD133+/CD44+ cells was elevated in SW1353 and SOSP-9607 cells after MALAT1 upregulation, while inhibition of MALAT1 reduced the proportion in osteosarcoma. Moreover, MALAT1 increased RET expression via sponging miR-129-5p and subsequently activated the Akt pathway in osteosarcoma cells. Another study showed that BMSC-EVs promoted the expression of MALAT1 and NRSN2, inhibited miR-143 expression, and triggered Wnt/b-catenin signaling pathway in osteosarcoma cells, resulting in enhancement of cell proliferation, invasion, and migration.

**The Functions of LncRNA PVT1 in Osteosarcoma Cells**

LncRNA PVT1 has been reported to positively govern the stemness of osteosarcoma cells. Specifically, stemness marker Nanog was highly expressed in osteosarcoma spheroids. ALDH1 activity and THOR expression were also remarkable in osteosarcoma spheroids. Overexpression of THOR elevated Nanog and ALDH1 expression, and increased ALDH1 activity, leading to improved spheroid size and number. Decreased expression of THOR caused the opposite effects in osteosarcoma spheroids. Down-regulation of THOR inhibited EMT and retarded the cell migratory capacity in OS spheroids. Mechanistically, THOR promoted the stability of SOX9 mRNA via directly interacting with SOX9 mRNA 3’ UTR. Furthermore, THOR facilitated the stemness of osteosarcoma cells via upregulation of SOX9 expression. Notably, depletion of THOR reduced cisplatin resistance of MG63 cell spheroids, indicating that THOR could contribute to cisplatin resistance in osteosarcoma cells. Consistent with this finding, THOR promoted the stem cell-like features via activating b-catenin signaling in TNBC cells and liver cancer cells. In brief, THOR is involved in the regulation of CSCs of human cancers.

**The Functions of LncRNA THOR in Osteosarcoma Cells**

LncRNA THOR has been reported to positively govern the stemness of osteosarcoma cells. THOR reduced cisplatin resistance of MG63 cell spheroids, indicating that THOR could contribute to cisplatin resistance in osteosarcoma cells. Consistent with this finding, THOR promoted the stem cell-like features via activating b-catenin signaling in TNBC cells and liver cancer cells. In brief, THOR is involved in the regulation of CSCs of human cancers.
promote gemcitabine resistance by suppressing miR-152 and activating the c-Met/PI3K/Akt pathway in osteosarcoma. Yan et al.
illustrated that PVT1 enhanced tumor metastasis by repressing miR-484 in osteosarcoma cells. Recently, it has been reported that PVT1 induced EMT through alteration of E-cadherin, N-cadherin, vimentin, and Snail expression in osteosarcoma cells. It also has been demonstrated that lncRNA PVT1 enhanced CSC traits in various types of human cancers. Cui et al.
found that lncRNA PVT1 facilitated CSC properties via suppressing miR-1207 and activating PI3K/Akt signaling pathway in nasopharyngeal cancer (NPC). Downregulation of PVT1 reduced the expression of Oct4, SOX2, ALDH, and c-Myc in NPC cells and impaired the stem phenotype. Wang et al.
indicated that lncRNA PVT1 contributed to stem cell-like traits via enhancement of NOP2 stability in HCC cells. Zhao et al.
reported that PVT1 encapsulated in BMSC-derived exosomes and stimulated cell growth and metastasis via acting as a miR-183-5p sponge and stabilizing ERG in osteosarcoma. The direct evidence for PVT1-mediated CSCs in osteosarcoma needs to be further investigated.

**The Functions of LncRNA H19 in Osteosarcoma Cells**

Accumulated evidence demonstrated that lncRNA H19 had a potential function in osteosarcoma development. LncRNA H19 acted as a ceRNA for reducing the activity of the miR-200 family and upregulating ZEB1 and ZEB2, leading to the promotion of tumor metastasis in osteosarcoma. He revealed that miR-141 inhibited cell proliferation and triggered apoptosis via downregulation of lncRNA H19 in osteosarcoma cells. Zhao and Ma.
reported that the inhibition of lncRNA H19 blocked cell migration and invasion via the NF-kB pathway in osteosarcoma. Interestingly, a recent study.
revealed that lncRNA H19 repressed osteosarcoma genesis via regulation of snoRNA7A expression and DNA damage repair protein complexes, showing a tumor suppressor role of lncRNA H19. H19 could affect several snoRNAs by analyzing H19-induced transcriptome changes in LFS iPSC-derived osteoblasts. LncRNA H19 promoted breast CSC properties via sponging let-7 and increasing LIN28 expression. Additionally, lncRNA H19 increased ERβ expression via sequestering miR-3126-5p, leading to the promotion of CSC traits in PTC. PIM protein kinases controlled H19 transcription by regulating the methylation of H19 promoter, leading to induction of SOX2, OCT-4, and Nanog in T-ALL cells. Strikingly, lncRNA H19 facilitated osteogenic differentiation by binding to miR-149 and elevating SDF-1 expression in BMSC cells. The role of lncRNA H19 in regulating CSCs in osteosarcoma is warranted to be deeper explored.

**The Functions of LncRNA B4GALT1-AS1 in Osteosarcoma Cells**

LncRNA B4GALT1-AS1 inhibited miR-30e and restored the expression of SOX9, and subsequently promoted cell proliferation in NSCLC. LncRNA B4GALT1-AS1 has been demonstrated to recruit YAP from cytoplasm to nucleus and increase YAP transcription, resulting in enhancement of colon cancer cell stemness. LncRNA B4GALT1-AS1 was highly elevated in osteosarcoma mamsospheres and tumor tissues. Depletion of B4GALT1-AS1 repressed proliferation, migration of osteosarcoma cells, and increased resistance of adriamycin. Moreover, depletion of B4GALT1-AS1 suppressed the expression of ALDH1 and Nanog, leading to reduction of spheres size and number, indicating that B4GALT1-AS1 was involved in sphere formation in osteosarcoma cells. Furthermore, the knockdown of B4GALT1-AS1 retarded osteosarcoma tumor growth in mice. Theoretically, B4GALT1-AS1 enhanced HuR nuclear-cytoplasmic translocation and promoted activation of YAP transcript in osteosarcoma cells.

**The Functions of LncRNA FER1L4 in Osteosarcoma Cells**

LncRNA FER1L4 has been characterized as a tumor suppressor in osteosarcoma. Lower expression of lncRNA FER1L4 was reported in osteosarcoma tissues. Clinically, FER1L4 expression was correlated with stage, metastasis, and tumor differentiation in osteosarcoma patients, suggesting that FER1L4 could be a biomarker for osteosarcoma prognosis. One study.
from China-Japan Union Hospital of Jilin University (Changchun, China) indicated that FER1L4 inhibited cell proliferation and motility and suppressed tumor growth in mice via interacting with miR-18a-5p and restoring the PTEN expression in osteosarcoma. Ye et al.
also found that downregulation of FER1L4 increased proliferation and suppressed apoptosis rate in osteo-


sarcoma cells. In addition, depletion of FER1L4 induced EMT, which was characterized by upregulation of N-cadherin, Vimentin, and Twist1 in osteosarcoma cells. Notably, knockdown of FER1L4 promoted the expression of CD133 and Nanog in osteosarcoma cells. Inconsistent, upregulation of FER1L4 displayed the opposite trends in osteosarcoma cells. Theoretically, the depletion of FER1L4 activated the expression of PI3K, pAkt via upregulation of miR-18a-5p and suppression of SOCS5 in osteosarcoma. In terms of the FER1L4 effect in CSC maintenance, another group from Beijing Friendship Hospital (Beijing, China) also found that FER1L4 blocked EMT and induced apoptosis by activation of PI3K/Akt pathway in osteosarcoma. Specifically, the increase of FER1L4 elevated E-cadherin and repressed fibronectin and vimentin, as well as inhibited the expression of several stemness markers, such as CD44, Oct4, SOX9, Nanog, and ALDH1 in osteosarcoma.

The Functions of LncRNA LINK-A in Osteosarcoma Cells

Several studies indicated that lncRNA LINK-A acted as an oncogene in a spectrum of human cancers, including NSCLC, ovarian cancer, pancreatic cancer, glioma, TNBC, and osteosarcoma. LncRNA LINK-A was demonstrated to accelerate cell migration and invasion via upregulation of HIF-1a and activation of TGF-b signaling pathway in ovarian cancer. Zhao et al. found that LINK-A expression in plasma was higher in metastatic osteosarcoma patients. LINK-A upregulation enhanced migration and invasion of MG-63 and U2OS cells via upregulation of HIF-1a. Another study demonstrated that plasma levels of lncRNA LINK-A were overexpressed in osteosarcoma patients. LINK-A was found to positively govern TGF-b1 expression in U2OS and MG-63 cells. In addition, LINK-A expression was associated with TGF-b1 expression in plasma of osteosarcoma patients. Downregulation of LINK-A suppressed migratory and invasive activity in osteosarcoma cells. LINK-A depletion reduced the percentage of CD133+ in U2OS and MG-63 cells, indicating that LINK-A could regulate the stemness of osteosarcoma cells.

The Functions of LncRNA DANC in Osteosarcoma Cells

The knockdown of DANC caused the downregulation of CSC markers, including ALDH1, CD44, and ABCG2, in MDA-MB-231 breast cancer cells via promoting the interaction of EZH2 with ABCG2 and CD44, and concomitant inhibition of their expressions. In A549 and H1755 NSCLC cells, DANC stimulated proliferation, invasion, and stemness via blocking miR-216a expression and inducing Wnt/b-catenin pathway. LncRNA DANC expression was highly expressed in osteosarcoma tissues and positively linked to tumor size and metastasis, suggesting that DANC might be a potential poor prognostic factor in osteosarcoma. Pan et al. discovered that silencing of DANC retarded SOX-5-induced osteosarcoma progression and autophagy via targeting miR-216a-5p. Zhang et al. found that DANC triggered cell migration and invasion via modulating miR-149 and MSI2 expression in osteosarcoma. Wang et al. also found that DANC enhanced ROCK-1-induced cell growth and lung metastasis via decoying miR-1972 and miR-335-5p in osteosarcoma. Ectopic expression of lncRNA DANC facilitated proliferation, migratory and invasive abilities of osteosarcoma cells, and accelerated tumor growth and lung metastasis in mice. Moreover, DANC enhanced CSC traits and osteosarcoma development. Theoretically, DANC elevated the expression of AXL via interacting with miR-33a-5p, leading to activation of PI3K/Akt pathway in osteosarcoma. Consistently, DANC expression was negatively associated with the amount of miR-33a-5p and positively correlated with AXL expression level in osteosarcoma patient tumor specimens.

The Functions of LncRNA DLX6-AS1 in Osteosarcoma Cells

LncRNA DLX6-AS1 has been reported to aggravate osteosarcoma stemness. The increased expression of lncRNA DLX6-AS1 existed in tumor tissues of osteosarcoma patients. Moreover, the DLX6-AS1 expression level was linked to tumor stage, poor grade, and poor overall survival in patients with osteosarcoma. Furthermore, DLX6-AS1 augmented osteosarcoma CSC properties, indicating that downregulation of DLX6-AS1 reduced sphere number and size, and decreased the proportions of the osteosarcoma cells stained with CD117 and Stro-1 positive. Molecular mechanistic investigations demonstrated that DLX6-AS1 elevated DLK1 via impairing miR-129a-5p interaction and activating the Wnt pathway.
Conclusions

Multiple IncRNAs involve in regulating CSCs in osteosarcoma via various molecular mechanisms (Figure 1). The targeting CSCs method for osteosarcoma therapy is an intriguing approach to improve the therapeutic outcomes, via the inhibition of metastasis and the promotion of drug sensitivity in osteosarcoma. However, several concerns appeared and needed to be addressed. For example, osteosarcoma could have a complex genomic landscape. It is difficult to use simple treatments for all osteosarcoma patients. It is known that many IncRNAs are involved in the maintenance of osteosarcoma stemness. However, it is not clear which IncRNAs are the key molecules to maintain the CSC properties in osteosarcoma cells. One IncRNA can sponge several miRNAs and subsequently increase the expression of multiple targets. It is uncertain how we could judge the key signaling pathways in controlling CSCs in osteosarcoma and how we could deliver the specific IncRNA inhibitors or mimics to bone tissue with osteosarcoma. Therefore, addressing the above issues will be certainly beneficial for us to develop new strategies for osteosarcoma treatment.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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References


The emerging role of lncRNAs in the regulation of osteosarcoma stem cells


