European Review for Medical and Pharmacological Sciences 2022; 26: 966-974

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

X.-J. SONG[1], M.-C. BI[2], Q.-S. ZHU[1], X.-L. LIU[3]

[1]Department of Orthopedic Surgery, China-Japan Union Hospital of Jilin University, Changchun, Jilin, China
[2]Department of Ophthalmology, The First Hospital of Jilin University, Changchun, Jilin, China
[3]Department of Hand Surgery, China-Japan Union Hospital of Jilin University, Changchun, Jilin, China

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, DANCR, 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; DANCR: 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 upstream 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.

Corresponding Authors: Qingsan Zhu, MD; e-mail: zhuqs@jlu.edu.cn
Xilin Liu, MD; e-mail: xilin2017@126.com

Introduction

Osteosarcoma, one of the most common bone malignancies, often occurs among juvenile or teenage patients[1]. 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[2]. 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[3,4].

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[5,6]. Evidence[7,8] 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[9-11]. Strikingly, targeting CSCs could overcome the drug resistance issue and inhibit the relapse and metastasis of osteosarcoma[12].

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[13,14]. 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[15]. LncRNAs perform their functions *via* serving as miRNA precursor, ceRNAs, and interacting with transcription preinitiation complex within the promoter[16,17]. LncRNAs have been demonstrated as potentially important factors during the development and progression process of osteosarcoma[18-20]. One research group from Xiangya Hospital has reported that several lncRNAs could be potential biomarkers for osteosarcoma prediction and treatment targets for osteosarcoma patients[21]. Notably, lncRNAs affect cisplatin resistance in osteosarcoma[22]. 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[23] have been reported that lncRNAs have a significant effect in regulating CSCs in osteosarcoma and other human cancers. Several studies[24-33] 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-AS1, FER1L4, LINK-A, DANCR, and DLX6-AS1. 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[26]. Ectopic expression of HIF2PUT reduced the number of CD133-expressing 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[26]. Theoretically, HIF2PUT exerted its biological functions *via* the promotion of HIF-2a expression in osteosarcoma cells[26]. Hereby, HIF2PUT expression was positively associated with HIF-2a levels in osteosarcoma specimens[26,34]. Zhao et al[24] 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[35]. 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[35]. Consistently, osteosarcoma patients with higher expression of HIF2PUT and HIF-2a exhibited an unfavorable prognosis[34]. HIF2PUT overexpression was correlated with tumor size, stage, distant metastasis, and overall survival (OS) and disease-free survival (DFS) in osteosarcoma patients[34]. 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[36]. LncRNA SOX2-OT was highly expressed in osteosarcoma specimens and several cell lines, such as SaOS-2, MG-63, U2OS, MNNG/HOS cell lines[25]. Moreover, lncRNA SOX2-OT expression was related to clinicopathological characteristics in patients with osteosarcoma, including tumor size, stage, histological grade, distant metastasis, and overall survival[25]. 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[25]. 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[37]. MALAT1 enhanced tumor metastasis and cell proliferation *via* serving as a ceRNA of miR-144-3p and inhibited ROCK1/ROCK2 expression in osteosarcoma cells[37]. MALAT1 overexpression was correlated with tumor invasion, metastasis, and prognosis in osteosarcoma patients[27]. 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[27]. 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 downregulation in SW1353 and SOSP-9607 cells[27]. 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[27]. Moreover, MALAT1 increased RET expression *via* sponging miR-129-5p and subsequently activated the Akt pathway in osteosarcoma cells[27]. Another study[38] 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 THOR in Osteosarcoma Cells

LncRNA THOR 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[28]. Moreover, 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[28]. Downregulation 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[28]. Notably, depletion of THOR reduced cisplatin resistance of MG63 cell spheroids, indicating that THOR could contribute to cisplatin resistance in osteosarcoma cells[28]. Consistent with this finding, THOR promoted the stem cell-like features via activating b-catenin signaling in TNBC cells and liver cancer cells[39,40]. In brief, THOR is involved in the regulation of CSCs of human cancers.

The Functions of LncRNA PVT1 in Osteosarcoma Cells

Emerging shreds of evidence[41-43] have been reported that lncRNA PVT1 regulated the proliferation, migration, invasion, and metastasis in osteosarcoma cells. For example, one group from Huai'an Hospital (Jiangsu, China) reported that PVT1 enhanced osteosarcoma development *via* sponging miR-195 in U2OS osteosarcoma cells[41]. Another group from Qingdao University (Qingdao, China) found that PVT1 enhanced glycolysis and osteosarcoma progression via sponging miR-497 and activating HK2[42]. In addition, PVT1 was shown to

promote gemcitabine resistance by suppressing miR-152 and activating the c-Met/PI3K/Akt pathway in osteosarcoma[43]. Yan et al[44] 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[45]. It also has been demonstrated that lncRNA PVT1 enhanced CSC traits in various types of human cancers[46,47]. Cui et al[47] 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[47]. Wang et al[46] indicated that lncRNA PVT1 contributed to stem cell-like traits *via* enhancement of NOP2 stability in HCC cells. Zhao et al[48] 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[49]. He revealed that miR-141 inhibited cell proliferation and triggered apoptosis *via* downregulation of lncRNA H19 in osteosarcoma cells[50]. Zhao and Ma[51] reported that the inhibition of lncRNA H19 blocked cell migration and invasion *via* the NF-kB pathway in osteosarcoma. Interestingly, a recent study[52] 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[52]. LncRNA H19 promoted breast CSC properties *via* sponging let-7 and increasing LIN28 expression[53]. Additionally, lncRNA H19 increased ERb expression *via* sequestering miR-3126-5p, leading to the promotion of CSC traits in PTC[54]. 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[55]. Strikingly, lncRNA H19 facilitated osteogenic differentiation by binding to miR-149 and elevating SDF-1 expression in BMSC cells[56]. 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[57]. 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[58]. LncRNA B4GALT1-AS1 was highly elevated in osteosarcoma mammospheres and tumor tissues. Depletion of B4GALT1-AS1 repressed proliferation, migration of osteosarcoma cells, and increased resistance of adriamycin[29]. 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 spheroid formation in osteosarcoma cells[29]. 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[29].

The Functions of LncRNA FER1L4 in Osteosarcoma Cells

LncRNA FER1L4 has been characterized as a tumor suppressor in osteosarcoma[59]. Lower expression of lncRNA FER1L4 was reported in osteosarcoma tissues[30,60]. Clinically, FER1L4 expression was correlated with stage, metastasis, and tumor differentiation in osteosarcoma patients, suggesting that FER1L4 could be a biomarker for osteosarcoma prognosis[60]. One study[59] 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[30] 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[30]. Notably, knockdown of FER1L4 promoted the expression of CD133 and Nanog in osteosarcoma cells[30]. 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[30]. 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[61]. 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[61].

The Functions of LncRNA LINK-A in Osteosarcoma Cells

Several studies[62-68] 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[69,70]. Zhao et al[63] 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[63]. Another study[31] 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[31]. Downregulation of LINK-A suppressed migratory and invasive activity in osteosarcoma cells[31]. 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[31].

The Functions of LncRNA DANCR in Osteosarcoma Cells

The knockdown of DANCR 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[71]. In A549 and H1755 NSCLC cells, DANCR stimulated proliferation, invasion, and stemness *via* blocking miR-216a expression and inducing Wnt/b-catenin pathway[72]. LncRNA DANCR expression was highly expressed in osteosarcoma tissues and positively linked to tumor size and metastasis, suggesting that DANCR might be a potential poor prognostic factor in osteosarcoma[32,73]. Pan et al[74] discovered that silencing of DANCR retarded SOX-5-induced osteosarcoma progression and autophagy *via* targeting miR-216a-5p. Zhang et al[75] found that DANCR triggered cell migration and invasion via modulating miR-149 and MSI2 expression in osteosarcoma. Wang et al[73] also found that DANCR enhanced ROCK-1-induced cell growth and lung metastasis via decoying miR-1972 and miR-335-5p in osteosarcoma. Ectopic expression of lncRNA DANCR facilitated proliferation, migratory and invasive abilities of osteosarcoma cells, and accelerated tumor growth and lung metastasis in mice[32]. Moreover, DANCR enhanced CSC traits and osteosarcoma development. Theoretically, DANCR elevated the expression of AXL *via* interacting with miR-33a-5p, leading to activation of PI3K/Akt pathway in osteosarcoma[32]. Consistently, DANCR expression was negatively associated with the amount of miR-33a-5p and positively correlated with AXL expression level in osteosarcoma patient tumor specimens[32].

The Functions of LncRNA DLX6-AS1 in Osteosarcoma Cells

LncRNA DLX6-AS1 has been reported to aggravate osteosarcoma stemness[33]. The increased expression of lncRNA DLX6-AS1 existed in tumor tissues of osteosarcoma patients[33]. Moreover, the DLX6-AS1 expression level was linked to tumor stage, poor grade, and poor overall survival in patients with osteosarcoma[33]. 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[33]. Molecular mechanistic investigations demonstrated that DLX6-AS1 elevated DLK1 via impairing miR-129a-5p interaction and activating the Wnt pathway[33].

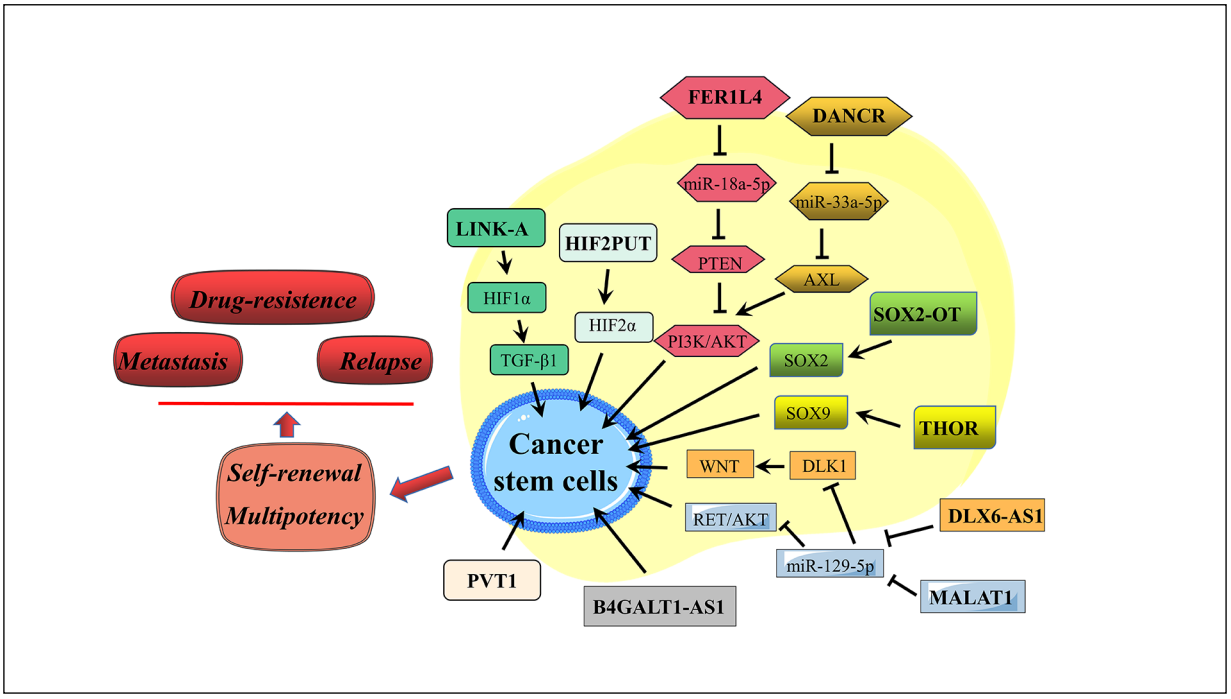

Figure 1. A diagram showing the role of lncRNAs in the regulation of OS stem cells.

Conclusions

Multiple lncRNAs 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 lncRNAs are involved in the maintenance of osteosarcoma stemness. However, it is not clear which lncRNAs are the key molecules to maintain the CSC properties in osteosarcoma cells. One lncRNA 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 lncRNA 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.

Funding

This work was supported by Science and Technology Department of Jilin Province (20180622005JC).

References

1) Gazouli I, Kyriazoglou A, Kotsantis I, Anastasiou M, Pantazopoulos A, Prevezanou M, Chatzidakis I, Kavourakis G, Economopoulou P, Kontogeorgakos V, Papagelopoulos P, Psyrri A. Systematic Review of Recurrent Osteosarcoma Systemic Therapy. Cancers (Basel) 2021; 13: 1757.
2) Meazza C Asaftei SD. State-of-the-art, approved therapeutics for the pharmacological management of osteosarcoma. Expert Opin Pharmacother 2021: 1-12.
3) Lallier M, Marchandet L, Moukengue B, Charrier C, Baud'huin M, Verrecchia F, Ory B, Lamoureux F. Molecular Chaperones in Osteosarcoma: Diagnosis and Therapeutic Issues. Cells 2021; 10: 754.
4) Tirtei E, Campello A, Asaftei SD, Mareschi K, Cereda M, Fagioli F. Precision Medicine in Osteosarcoma: MATCH Trial and Beyond. Cells 2021; 10: 281.

5) Bayik D Lathia JD. Cancer stem cell-immune cell crosstalk in tumour progression. Nat Rev Cancer 2021; 21: 526-536.
6) Lim JR, Mouawad J, Gorton OK, Bubb WA, Kwan AH. Cancer stem cell characteristics and their potential as therapeutic targets. Med Oncol 2021; 38: 76.
7) Basu-Roy U, Basilico C, Mansukhani A. Perspectives on cancer stem cells in osteosarcoma. Cancer Lett 2013; 338: 158-167.
8) Schiavone K, Garnier D, Heymann MF, Heymann D. The Heterogeneity of Osteosarcoma: The Role Played by Cancer Stem Cells. Adv Exp Med Biol 2019; 1139: 187-200.
9) Yan GN, Lv YF, Guo QN. Advances in osteosarcoma stem cell research and opportunities for novel therapeutic targets. Cancer Lett 2016; 370: 268-274.
10) Brown HK, Tellez-Gabriel M, Heymann D. Cancer stem cells in osteosarcoma. Cancer Lett 2017; 386: 189-195.
11) Belayneh R Weiss K. The Role of ALDH in the Metastatic Potential of Osteosarcoma Cells and Potential ALDH Targets. Adv Exp Med Biol 2020; 1258: 157-166.
12) Izadpanah S, Shabani P, Aghebati-Maleki A, Baghbanzadeh A, Fotouhi A, Bisadi A, Aghebati-Maleki L, Baradaran B. Prospects for the involvement of cancer stem cells in the pathogenesis of osteosarcoma. J Cell Physiol 2020; 235: 4167-4182.
13) Borkiewicz L, Kalafut J, Dudziak K, Przybyszewska-Podstawka A, Telejko I. Decoding LncRNAs. Cancers (Basel) 2021; 13: 2643.
14) Liu SJ, Dang HX, Lim DA, Feng FY, Maher CA. Long noncoding RNAs in cancer metastasis. Nat Rev Cancer 2021; 21: 446-460.
15) Statello L, Guo CJ, Chen LL, Huarte M. Gene regulation by long non-coding RNAs and its biological functions. Nat Rev Mol Cell Biol 2021; 22: 96-118.
16) Nair L, Chung H, Basu U. Regulation of long non-coding RNAs and genome dynamics by the RNA surveillance machinery. Nat Rev Mol Cell Biol 2020; 21: 123-136.
17) Fatica A Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. Nat Rev Genet 2014; 15: 7-21.
18) Wang JY, Yang Y, Ma Y, Wang F, Xue A, Zhu J, Yang H, Chen Q, Chen M, Ye L, Wu H, Zhang Q. Potential regulatory role of lncRNA-miRNA-mRNA axis in osteosarcoma. Biomed Pharmacother 2020; 121: 109627.
19) Han J Shen X. Long noncoding RNAs in osteosarcoma via various signaling pathways. J Clin Lab Anal 2020; 34: e23317.
20) Ghafouri-Fard S, Shirvani-Farsani Z, Hussen BM, Taheri M. The critical roles of lncRNAs in the development of osteosarcoma. Biomed Pharmacother 2021; 135: 111217.
21) Li Z, Dou P, Liu T, He S. Application of Long Noncoding RNAs in Osteosarcoma: Biomarkers and Therapeutic Targets. Cell Physiol Biochem 2017; 42: 1407-1419.
22) Ferretti VA Leon IE. Long Non-coding RNAs in Cisplatin Resistance in Osteosarcoma. Curr Treat Options Oncol 2021; 22: 41.
23) Castro-Oropeza R, Melendez-Zajgla J, Maldonado V, Vazquez-Santillan K. The emerging role of lncRNAs in the regulation of cancer stem cells. Cell Oncol (Dordr) 2018; 41: 585-603.
24) Zhao D, Wang S, Chu X, Han D. LncRNA HIF-2PUT inhibited osteosarcoma stem cells proliferation, migration and invasion by regulating HIF2 expression. Artif Cells Nanomed Biotechnol 2019; 47: 1342-1348.
25) Wang Z, Tan M, Chen G, Li Z, Lu X. LncRNA SOX2-OT is a novel prognostic biomarker for osteosarcoma patients and regulates osteosarcoma cells proliferation and motility through modulating SOX2. IUBMB Life 2017; 69: 867-876.
26) Wang Y, Yao J, Meng H, Yu Z, Wang Z, Yuan X, Chen H, Wang A. A novel long non-coding RNA, hypoxia-inducible factor-2alpha promoter upstream transcript, functions as an inhibitor of osteosarcoma stem cells in vitro. Mol Med Rep 2015; 11: 2534-2540.
27) Chen Y, Huang W, Sun W, Zheng B, Wang C, Luo Z, Wang J, Yan W. LncRNA MALAT1 Promotes Cancer Metastasis in Osteosarcoma via Activation of the PI3K-Akt Signaling Pathway. Cell Physiol Biochem 2018; 51: 1313-1326.
28) Wu H, He Y, Chen H, Liu Y, Wei B, Chen G, Lin H, Lin H. LncRNA THOR increases osteosarcoma cell stemness and migration by enhancing SOX9 mRNA stability. FEBS Open Bio 2019; 9: 781-790.
29) Li Z, Wang Y, Hu R, Xu R, Xu W. LncRNA B4GALT1-AS1 recruits HuR to promote osteosarcoma cells stemness and migration via enhancing YAP transcriptional activity. Cell Prolif 2018; 51: e12504.
30) Ye F, Tian L, Zhou Q, Feng D. LncRNA FER1L4 induces apoptosis and suppresses EMT and the activation of PI3K/AKT pathway in osteosarcoma cells via inhibiting miR-18a-5p to promote SOCS5. Gene 2019; 721: 144093.
31) Kong Y, Nie Z, Guo H, Ma C. LINK-A lncRNA is upregulated in osteosarcoma and regulates migration, invasion and stemness of osteosarcoma cells. Oncol Lett 2020; 19: 2832-2838.
32) Jiang N, Wang X, Xie X, Liao Y, Liu N, Liu J, Miao N, Shen J, Peng T. lncRNA DANCR promotes tumor progression and cancer stemness features in osteosarcoma by upregulating AXL via miR-33a-5p inhibition. Cancer Lett 2017; 405: 46-55.
33) Zhang RM, Tang T, Yu HM, Yao XD. LncRNA DLX6-AS1/miR-129-5p/DLK1 axis aggravates stemness of osteosarcoma through Wnt signaling. Biochem Biophys Res Commun 2018; 507: 260-266.

34) Li W, He X, Xue R, Zhang Y, Zhang X, Lu J, Zhang Z, Xue L. Combined over-expression of the hypoxia-inducible factor 2alpha gene and its long non-coding RNA predicts unfavorable prognosis of patients with osteosarcoma. Pathol Res Pract 2016; 212: 861-866.

35) Yao J, Li J, Geng P, Li Y, Chen H, Zhu Y. Knockdown of a HIF-2alpha promoter upstream long noncoding RNA impairs colorectal cancer stem cell properties in vitro through HIF-2alpha downregulation. Onco Targets Ther 2015; 8: 3467-74.

36) Li PY, Wang P, Gao SG, Dong DY. Long Noncoding RNA SOX2-OT: Regulations, Functions, and Roles on Mental Illnesses, Cancers, and Diabetic Complications. Biomed Res Int 2020; 2020: 2901589.

37) Wang Y, Zhang Y, Yang T, Zhao W, Wang N, Li P, Zeng X, Zhang W. Long non-coding RNA MALAT1 for promoting metastasis and proliferation by acting as a ceRNA of miR-144-3p in osteosarcoma cells. Oncotarget 2017; 8: 59417-59434.

38) Li F, Chen X, Shang C, Ying Q, Zhou X, Zhu R, Lu H, Hao X, Dong Q, Jiang Z. Bone Marrow Mesenchymal Stem Cells-Derived Extracellular Vesicles Promote Proliferation, Invasion and Migration of Osteosarcoma Cells via the lncRNA MALAT1/miR-143/NRSN2/Wnt/beta-Catenin Axis. Onco Targets Ther 2021; 14: 737-749.

39) Wang B, Ye Q, Zou C. Long Non-Coding RNA THOR Enhances the Stem Cell-Like Traits of Triple-Negative Breast Cancer Cells Through Activating beta-Catenin Signaling. Med Sci Monit 2020; 26: e923507.

40) Cheng Z, Lei Z, Yang P, Si A, Xiang D, Zhou J, Huser N. Long non-coding RNA THOR promotes liver cancer stem cells expansion via beta-catenin pathway. Gene 2019; 684: 95-103.

41) Zhou Q, Chen F, Zhao J, Li B, Liang Y, Pan W, Zhang S, Wang X, Zheng D. Long non-coding RNA PVT1 promotes osteosarcoma development by acting as a molecular sponge to regulate miR-195. Oncotarget 2016; 7: 82620-82633.

42) Song J, Wu X, Liu F, Li M, Sun Y, Wang Y, Wang C, Zhu K, Jia X, Wang B, Ma X. Long non-coding RNA PVT1 promotes glycolysis and tumor progression by regulating miR-497/HK2 axis in osteosarcoma. Biochem Biophys Res Commun 2017; 490: 217-224.

43) Sun ZY, Jian YK, Zhu HY, Li B. lncRNAPVT1 targets miR-152 to enhance chemoresistance of osteosarcoma to gemcitabine through activating c-MET/PI3K/AKT pathway. Pathol Res Pract 2019; 215: 555-563.

44) Yan M, Pan XF, Liu Y, Zhao S, Gong WQ, Liu W. Long noncoding RNA PVT1 promotes metastasis via miR-484 sponging in osteosarcoma cells. Eur Rev Med Pharmacol Sci 2020; 24: 2229-2238.

45) Xun C, Jiang D, Tian Z, Yunus A, Chen J. Long noncoding RNA plasmacytoma variant translocation gene 1 promotes epithelial-mesenchymal transition in osteosarcoma. J Clin Lab Anal 2021; 35: e23587.

46) Wang F, Yuan JH, Wang SB, Yang F, Yuan SX, Ye C, Yang N, Zhou WP, Li WL, Li W, Sun SH. Oncofetal long noncoding RNA PVT1 promotes proliferation and stem cell-like property of hepatocellular carcinoma cells by stabilizing NOP2. Hepatology 2014; 60: 1278-1290.

47) Cui M, Chang Y, Fang QG, Du W, Wu JF, Wang JH, Liu ST, Luo SX. Non-Coding RNA Pvt1 Promotes Cancer Stem Cell-Like Traits in Nasopharyngeal Cancer via Inhibiting miR-1207. Pathol Oncol Res 2019; 25: 1411-1422.

48) Zhao W, Qin P, Zhang D, Cui X, Gao J, Yu Z, Chai Y, Wang J, Li J. Long non-coding RNA PVT1 encapsulated in bone marrow mesenchymal stem cell-derived exosomes promotes osteosarcoma growth and metastasis by stabilizing ERG and sponging miR-183-5p. Aging (Albany NY) 2019; 11: 9581-9596.

49) Li M, Chen H, Zhao Y, Gao S, Cheng C. H19 Functions as a ceRNA in Promoting Metastasis Through Decreasing miR-200s Activity in Osteosarcoma. DNA Cell Biol 2016; 35: 235-240.

50) He P, Zhang Z, Huang G, Wang H, Xu D, Liao W, Kang Y. miR-141 modulates osteoblastic cell proliferation by regulating the target gene of lncRNA H19 and lncRNA H19-derived miR-675. Am J Transl Res 2016; 8: 1780-1788.

51) Zhao J, Ma ST. Downregulation of lncRNA H19 inhibits migration and invasion of human osteosarcoma through the NF-kappaB pathway. Mol Med Rep 2018; 17: 7388-7394.

52) Xu A, Huang MF, Zhu D, Gingold JA, Bazer DA, Chang B, Wang D, Lai CC, Lemischka IR, Zhao R, Lee DF. LncRNA H19 Suppresses Osteosarcomagenesis by Regulating snoRNAs and DNA Repair Protein Complexes. Front Genet 2020; 11: 611823.

53) Peng F, Li TT, Wang KL, Xiao GQ, Wang JH, Zhao HD, Kang ZJ, Fan WJ, Zhu LL, Li M, Cui B, Zheng FM, Wang HJ, Lam EW, Wang B, Xu J, Liu Q. H19/let-7/LIN28 reciprocal negative regulatory circuit promotes breast cancer stem cell maintenance. Cell Death Dis 2017; 8: e2569.

54) Li M, Chai HF, Peng F, Meng YT, Zhang LZ, Zhang L, Zou H, Liang QL, Li MM, Mao KG, Sun DX, Tong MY, Deng ZQ, Hou ZJ, Zhao Y, Li J, Wang XC, Lv SS, Zhang QQ, Yu X, Lam EW, Liu Q, Cui XN, Xu J. Estrogen receptor beta upregulated by lncRNA-H19 to promote cancer stem-like properties in papillary thyroid carcinoma. Cell Death Dis 2018; 9: 1120.

55) Singh N, Padi SKR, Bearss JJ, Pandey R, Okumura K, Beltran H, Song JH, Kraft AS, Olive V. PIM protein kinases regulate the level of the long noncoding RNA H19 to control stem cell gene transcription and modulate tumor growth. Mol Oncol 2020; 14: 974-990.

56) Li G, Yun X, Ye K, Zhao H, An J, Zhang X, Han X, Li Y, Wang S. Long non-coding RNA-H19

stimulates osteogenic differentiation of bone marrow mesenchymal stem cells via the microRNA-149/SDF-1 axis. J Cell Mol Med 2020; 24: 4944-4955.

57) Lin JH, Chen FN, Wu CX, Hu SQ, Ma J. Long non-coding RNA B4GALT1-Antisense RNA 1/microRNA-30e/SRY-box transcription factor 9 signaling axis contributes to non-small cell lung cancer cell growth. Oncol Lett 2020; 20: 284.

58) Zhang Y, Fang Z, Guo X, Dong H, Zhou K, Huang Z, Xiao Z. lncRNA B4GALT1-AS1 promotes colon cancer cell stemness and migration by recruiting YAP to the nucleus and enhancing YAP transcriptional activity. J Cell Physiol 2019; 234: 18524-18534.

59) Fei D, Zhang X, Liu J, Tan L, Xing J, Zhao D, Zhang Y. Long Noncoding RNA FER1L4 Suppresses Tumorigenesis by Regulating the Expression of PTEN Targeting miR-18a-5p in Osteosarcoma. Cell Physiol Biochem 2018; 51: 1364-1375.

60) Chen ZX, Chen CP, Zhang N, Wang TX. Low-expression of lncRNA FER1L4 might be a prognostic marker in osteosarcoma. Eur Rev Med Pharmacol Sci 2018; 22: 2310-2314.

61) Ma L, Zhang L, Guo A, Liu LC, Yu F, Diao N, Xu C, Wang D. Overexpression of FER1L4 promotes the apoptosis and suppresses epithelial-mesenchymal transition and stemness markers via activating PI3K/AKT signaling pathway in osteosarcoma cells. Pathol Res Pract 2019; 215: 152412.

62) Maleki P, Mowla SJ, Taheri M, Ghafouri-Fard S, Raheb J. The role of long intergenic non-coding RNA for kinase activation (LINK-A) as an oncogene in non-small cell lung carcinoma. Sci Rep 2021; 11: 4210.

63) Zhao B, Liu K, Cai L. LINK-A lncRNA functions in the metastasis of osteosarcoma by upregulating HIF1alpha. Oncol Lett 2019; 17: 5005-5011.

64) Maleki P, Sheida SV, Mowla SJ, Soleimani V, Taheri M, Raheb J. LINK-A long non-coding RNA and VEGF RNA expression in epithelial ovarian cancer patients. Hum Antibodies 2020; 28: 227-232.

65) Zhang M, Wang R, Zhao X, Lu L, Wang T. LncRNA LINK-A regulates ROCK1 expression in early-stage pancreatic adenocarcinoma. Exp Ther Med 2020; 19: 1933-1939.

66) Liu J, Song W, Li J, Li X, Zhao R, Gong T. LINK-A lncRNA is upregulated in metastatic non-small cell lung cancer and is associated with poor prognosis. Oncol Lett 2019; 18: 3049-3057.

67) Hua X, Li G, Liu Z, Niu Z. LINK-A lncRNA participates in the pathogenesis of glioma by interacting with survivin. Exp Ther Med 2019; 18: 1581-1586.

68) Lin A, Li C, Xing Z, Hu Q, Liang K, Han L, Wang C, Hawke DH, Wang S, Zhang Y, Wei Y, Ma G, Park PK, Zhou J, Zhou Y, Hu Z, Zhou Y, Marks JR, Liang H, Hung MC, Lin C, Yang L. The LINK-A lncRNA activates normoxic HIF1alpha signalling in triple-negative breast cancer. Nat Cell Biol 2016; 18: 213-224.

69) Ma J, Xue M. LINK-A lncRNA promotes migration and invasion of ovarian carcinoma cells by activating TGF-beta pathway. Biosci Rep 2018; 38: BSR20180936.

70) Zhang H, Yao B, Tang S, Chen Y. LINK-A Long Non-Coding RNA (lncRNA) Participates in Metastasis of Ovarian Carcinoma and Upregulates Hypoxia-Inducible Factor 1 (HIF1alpha). Med Sci Monit 2019; 25: 2221-2227.

71) Sha S, Yuan D, Liu Y, Han B, Zhong N. Targeting long non-coding RNA DANCR inhibits triple negative breast cancer progression. Biol Open 2017; 6: 1310-1316.

72) Yu JE, Ju JA, Musacchio N, Mathias TJ, Vitolo MI. Long Noncoding RNA DANCR Activates Wnt/beta-Catenin Signaling through MiR-216a Inhibition in Non-Small Cell Lung Cancer. Biomolecules 2020; 10: 1646.

73) Wang Y, Zeng X, Wang N, Zhao W, Zhang X, Teng S, Zhang Y, Lu Z. Long noncoding RNA DANCR, working as a competitive endogenous RNA, promotes ROCK1-mediated proliferation and metastasis via decoying of miR-335-5p and miR-1972 in osteosarcoma. Mol Cancer 2018; 17: 89.

74) Pan Z, Wu C, Li Y, Li H, An Y, Wang G, Dai J, Wang Q. LncRNA DANCR silence inhibits SOX5-medicated progression and autophagy in osteosarcoma via regulating miR-216a-5p. Biomed Pharmacother 2020; 122: 109707.

75) Zhang W, Li JZ, Tai QY, Tang JJ, Huang YH, Gao SB. LncRNA DANCR regulates osteosarcoma migration and invasion by targeting miR-149/MSI2 axis. Eur Rev Med Pharmacol Sci 2020; 24: 6551-6560.