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Long Noncoding RNAs in Osteosarcoma: Mechanisms and Potential Clinical Implications

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Abstract

Long noncoding RNAs (lncRNAs) are noncoding transcripts consisting of a diverse class of long RNAs of more than 200 nucleotides in length. Recent studies have shown that lncRNAs are involved in cell signal transduction pathways, cell cycle and cell death regulation, chromatin remodeling, and gene expression regulation at the transcriptional and posttranscriptional levels. They are also involved in the metastatic process of different types of tumors, such as urothelial carcinoma, colon carcinoma, breast carcinoma, lung carcinoma, and hepatocellular carcinoma. In addition, lncRNAs demonstrate precise expression patterns in specific tissues and cells and therefore play important roles in cell differentiation and tissue development. In this chapter, we review the molecular mechanisms of lncRNA cell functions and their involvement in the pathogenesis, progression, and metastasis of osteosarcoma, a rare bone tumor of childhood and adolescence. We also review emerging clinical implications of lncRNA use as potential prognostic biomarkers and therapeutic targets, as well as their putative involvement in drug resistance, in osteosarcoma progression, and in therapeutic interventions.

Keywords: lncRNAs, osteosarcoma, pathogenesis, prognosis, metastasis, drug resistance

1. Introduction

Osteosarcoma is a rare malignant tumor and the most frequent primary malignant tumor of the bone affecting most often young people in childhood and adolescence [1–3]. It is of mesenchymal histogenetic origin and is characterized by the production of osteoid and fibrous stroma. It has a tendency to be highly anaplastic with cytological pleomorphism consisting of cells of epithelioid, spindle, ovoid, or giant multinucleated appearance and in most cases a mixture of them [4]. It is genetically unstable and exhibits structural chromosomal alterations [5–8]. It represents different pathological entities based on clinical, radiological, and histopathological features. Depending on histopathological features, osteosarcoma displays different subtypes, the most common among them are osteoblastic osteosarcoma, chondroblastic osteosarcoma, and fibroblastic osteosarcoma. Less frequent are telangiectatic osteosarcoma, small cell osteosarcoma, low-grade osteosarcoma, high-grade osteosarcoma, parosteal osteosarcoma, and periosteal osteosarcoma [4, 9–11].

Its incidence is about three to five cases per million population every year worldwide with a propensity of aggressive biological behavior, local infiltrating growth, and distant metastasis [1–3]. About 10–25% of patients are diagnosed with pulmonary metastasis due to hematogenous dissemination, which is the main cause of osteosarcoma mortality [12–14].

Despite its high mortality rates, the combination of ablative resection surgery with chemotherapy or/and radiation therapy has elevated the cure rates of local tumor from less than 20% during 1960s to 65–75% at present days [12–17]. However, patients with disseminated disease demonstrate a 5-year survival rate around 11–30% due to resistance to chemotherapeutic regimens [16–18]. Therefore, developing multimodal more effective treatments along with precise prognostic and preventive biomarkers is imperative, and efforts are on the way to better understand the molecular mechanisms involved in osteosarcoma pathogenesis and define new therapeutic targets.

Recent studies have shown that molecules belonging to the nonprotein-coding transcriptome may play essential roles in a wide range of biological processes [19–21]. These molecules belong to the vast family of nonprotein-coding RNAs which can be classified according to their size or function in two classes: the short noncoding RNAs (sncRNAs) and the long noncoding RNAs (lncRNAs) [22, 23].

Short noncoding RNAs, with a length less than 200 nucleotides, such as microRNAs (miRNAs), transfer RNAs, small interfering RNAs (siRNAs), piwi-interacting RNAs, and some ribosomal RNAs, are estimated to be, till now, about 2500 different types. They are involved in gene expression regulation and have been demonstrated to play important roles in cancer development, progression, and chemoresistance of different tumors including osteosarcoma [22, 23].

On the other hand, lncRNAs are noncoding transcripts consisting of a diverse and heterogeneous class of long RNAs of more than 200 nucleotides –100 kb in length lacking the Kozak consensus sequence and without open reading frame. Their transcription is processed through RNA polymerase II and is regulated by the transcriptional activators of the nucleosome remodeling complex SWI/SNF [23–26]. They are divided in different categories such as intronic lncRNAs, intergenic lncRNAs, UTR-associated lncRNAs, bidirectional lncRNAs, promoter-associated lncRNAs, sense lncRNAs, and antisense lncRNAs [27, 28]. They participate in vital biological processes, such as cell signal transduction, cell cycle and cell death regulation, chromatin remodeling, transcriptional and posttranscriptional processing, as well as in epigenetic gene regulation. They can act as decoys to compete with different proteins, function as sponge to a large number of microRNAs, and interact with RNA-binding proteins. In addition, lncRNAs demonstrate precise expression patterns in specific tissues and cells and therefore play important roles in cell differentiation and tissue development. [29–31]. LncRNA misregulation has been implicated in cancer development, metastatic process, and drug resistance of different types of tumors, such as urothelial carcinoma, colon carcinoma, breast carcinoma, and hepatocellular carcinoma. Aberrant expression of lncRNAs has been seen in different human tumors, an observation that might be exploited for diagnostic, prognostic, preventive, or therapeutic purposes [32–41]. Some of these lncRNAs have also been reported to play a crucial role in osteosarcoma pathogenesis and metastatic process as well as in chemotherapy drug resistance. Thus, they are considered candidate molecules as prognostic or preventive biomarkers and/or novel therapeutic targets [42–47].

In this chapter, we review the molecular mechanisms of lncRNA cell functions and their involvement in the pathogenesis, progression, and metastasis of osteosarcoma. We also review emerging clinical implications of lncRNA use as potential

prognostic biomarkers and therapeutic targets, as well as their putative involvement in drug resistance, in osteosarcoma progression, and in therapeutic interventions.

2. LncRNAs and signal transduction pathways in osteosarcoma

Osteosarcomagenesis is initiated in bone epiphyseal growth plates with rapid turnover during childhood and adolescence and has also been observed in high incidence in patients affected by Paget's disease, a pathological entity characterized by excessive osteoid formation and breakdown. These findings suggest that molecular disturbances in osteoblast proliferation and differentiation are involved in osteosarcoma pathogenesis through dysregulation of major signal transduction pathways and osteogenic transcriptional factors [4, 42–47]. Several major signal transduction pathways, mainly Wnt/ β -catenin, bone morphogenetic protein (BMP), Hedgehog, HIF1 α , Notch, PI3K/Akt, JNK and NF- κ B pathways are implicated in osteosarcoma development and metastatic progression [48–50].

The canonical Wnt/ β -catenin pathway, which plays a crucial role in osteoblast differentiation, has been found to lead to osteoblast proliferation and suppression of osteogenic differentiation in adult mesenchymal cells through expression of Wnt3a [51–54]. Moreover, aberrations of Wnt signaling pathway have been associated with osteosarcoma tumorigenesis and osteosarcoma drug resistance through upregulation of factors, such as c-Met, leading to stem-cell phenotypes [4, 55–58]. LncRNA H19 has been found to increase Wnt signaling through epigenetical regulation of the Wnt pathway antagonist NKD1 via EZH2 recruitment [59]. Wnt pathway is also activated through TCF7 whose expression is triggered by the recruitment of SWI/SNF nucleosome remodeling complex to the TCF7 promoter by lncTCF7 [60, 61].

Hedgehog (Hh) signaling pathway plays a crucial role during vertebrate embryogenesis acting as a morphogen and mitogen in different tissue development including bone morphogenesis [62–66].

Dysregulation of Hh signaling pathway has been demonstrated to contribute to promigratory effects in osteoblastic osteosarcoma and is related to poor prognosis [67, 68]. Moreover Hedgehog signaling is upregulated in osteosarcoma leading to overexpression of oncogenic yes-associated protein 1 (Yap1) which in turn induces the aberrant expression of lncRNA H19 [69].

Bone morphogenetic protein (BMP) signaling pathways synergistically act with Runx2 factor, the most important regulator of bone development, leading to the induction of many terminal differentiation factors and eventually to osteogenic commitment of mesenchymal stem cells. This signaling cascade is initiated by BMP ligand heterodimers (BMP I and II) binding through Smad and mitogen-activated protein kinase (MAPK) phosphorylation [70–73]. Suppression of osteoblast differentiation has been observed, in one study, after BMP2 treatment of C3H10T1/2 MSCs by downregulation of mouse lncRNA0231 and EGFR via Runx2 and osterix regulation [74]. In another study, anti-differentiation lncRNA (ANCR) has been found to suppress osteoblastogenesis through inhibition of Runx2 expression. ANCR interacts with the enhancer of zeste homolog 2 (EZH2); this interaction leads to H3K27me3 catalysis in Runx2 promoter resulting in inhibition of Runx2 expression [75]. Bone morphogenetic protein (BMP) signaling pathways play also an important role in osteosarcoma through RhoA-ROCK-LIMK2 by promoting invasion and metastasis [76, 77].

HIF1 α expression levels are elevated in osteosarcoma tissues and are associated with poorer prognosis. Moreover, HIF1 α signaling pathway is implicated in osteosarcoma cell invasion through induction of VEGF-A expression [78, 79]. A novel

lncRNA, hypoxia-inducible factor-2 α (HIF-2 α) promoter upstream transcript (HIF2PUT) has been demonstrated to regulate the expression of HIF-2 α in osteosarcoma stem cells. Overexpression of HIF2PUT significantly inhibited cell proliferation and migration of MG63 osteosarcoma cells, while HIF2PUT knockdown led to the opposite effect [80].

lncRNA hypoxia-inducible factor 1 α -antisense 1 (HIF1 α -AS1) is another lncRNA involved in osteoblast differentiation. HIF1 α -AS1 expression is repressed by overexpression of histone deacetylase sirtuin 1 (SIRT1), a regulator of osteoblastogenesis, and lower levels of SIRT1 expression lead to upregulation of HIF1 α -AS1 in bone marrow stem cells resulting in the activation of osteoblastogenesis [81].

Other studies have also shown the involvement of Notch and JNK signaling pathways in osteosarcoma proliferation, metastasis, angiogenesis, and stemness-associated factors [82, 83].

The phosphatidylinositol 3-kinase PI3K/Akt pathway is considered one of the most critical pathways in osteosarcoma pathogenesis regulating osteosarcoma cell proliferation, invasion, metastasis, and drug sensitivity or resistance [84, 85].

A large number of lncRNAs has been found to be differentially expressed in osteosarcoma either with oncogenic or tumor suppressive activity. Particularly, in a study by Li et al., 25,733 lncRNAs were detected, including 403 constitutively upregulated in 34 pathways and 798 constitutively downregulated in 32 pathways (twofold, $P < 0.05$) [86]. Among them metastasis-associated lung adenocarcinoma transcript 1 (MALAT-1), a lncRNA involved in regulating the recruitment of pre-mRNA-splicing factors to transcription sites, is overexpressed in osteosarcoma, and its expression level is highly related to the metastatic potential of the tumor. In another study, Dong et al. also found that MALAT-1 acts through the PI3K/Akt pathway to promote osteosarcoma cell proliferation, migration, invasion, and pulmonary metastasis [87]. MALAT-1 knockdown or siRNA interference experiments, carried out by Dong et al. and Cai et al., respectively, showed that MALAT1 inhibition suppressed osteosarcoma cell proliferation and metastasis via the PI3K/Akt and RhoA/ROCK signaling pathway by decreasing the expression levels of proliferating cell nuclear antigen (PCNA), Act and phosphorylated PI3Kp85 α , as well as MMP-9 metalloproteinase [87, 88].

Another lncRNA, named P50-associated COX-2 extragenic RNA (PACER), has been found to be overexpressed in osteosarcoma clinical specimens and cell lines. PACER has oncogenic effects in osteosarcoma functioning by activating COX-2 gene via the NF- κ B signaling cascade [89]. Deregulated NF- κ B has been linked to osteosarcoma cell proliferation and metastatic process, and expression of NF- κ B has been observed to have clinical value in osteosarcoma patients [90, 91].

3. lncRNAs and regulation of cell growth/proliferation in osteosarcoma

Recent studies have demonstrated the involvement of lncRNAs in cell growth and proliferation of osteosarcoma. Aberrant expression of lncRNAs is implicated in osteosarcoma tumorigenesis through overexpression of oncogenic lncRNAs and inhibition of tumor suppressive lncRNAs [42–44, 92]. These lncRNAs are summarized in **Table 1** along with their function and mechanisms.

3.1 Oncogenic lncRNAs

In recent years, a significant number of oncogenic lncRNAs such as 91H, HULC, FGFR3-AS1, MALAT1, BCAR4, HIF2PUT, TUG1, UCA1, HOTTIP, and HOTAIR have been identified to be implicated in cell growth and proliferation of osteosarcoma.

lncRNA	Chr. locus	Transcript length	Expression	Function	Mechanisms	Refs
91H (H19)	11p15.5	2.3 kb	Upregulated	<ul style="list-style-type: none"> • Oncogenic • Promotes cell proliferation • Reduced levels • Promote apoptosis 	<ul style="list-style-type: none"> • IGF2 transcriptional regulation • Imprinted gene • miR-141 overexpression leads to OS • Apoptosis through suppression of H19 	[94, 95, 197]
BANCR	9q21.11	693 bp	Upregulated	<ul style="list-style-type: none"> • Promotes tumor growth, invasion, and metastasis 	—	[198]
BCAR4	16p13.13	118 bp	Upregulated	<ul style="list-style-type: none"> • Oncogenic • Promotes cell proliferation 	<ul style="list-style-type: none"> • Activation of GLI2-dependent gene transcription 	[104, 105]
DANCR (ANCR)	4q12	855 bp	Upregulated	<ul style="list-style-type: none"> • Oncogenic • Suppresses osteogenic differentiation • Promotes cell proliferation and metastasis 	<ul style="list-style-type: none"> • Decoy for miR-335-5p and miR-1972 • Inhibits Runx2 expression • Interacts with enhancer of EZH2 • Regulates the expression of p21, CDK2, and CDK4 	[100, 101, 207]
FGFR3-AS1	4p16.3	—	Upregulated	<ul style="list-style-type: none"> • Oncogenic • Promotes cell proliferation 	<ul style="list-style-type: none"> • Increases FGFR3 mRNA stability • Increases FGFR3 expression 	[107]
HIF2PUT	2p21		Upregulated	<ul style="list-style-type: none"> • Oncogenic 	<ul style="list-style-type: none"> • Involvement in HIF-2a and stemness-related genes (Oct4, Sox, CD44) expression 	[80, 112]
HOTAIR	12q13.13	2337 bp	Upregulated	<ul style="list-style-type: none"> • Oncogenic • Promotes cell proliferation, invasion, and metastasis 	<ul style="list-style-type: none"> • Inhibits gene expression through histone H3K27 trimethylation by binding PRC2 and LSD1/CoREST/REST complexes • Upregulation of MMP-2 and MMP-9 	[118, 119, 207]
HOTTIP	7p15.2	4.6 kb	Upregulated	<ul style="list-style-type: none"> • Oncogenic • Promotes cell proliferation, invasion, and metastasis 	<ul style="list-style-type: none"> • Regulates EMT-related molecules (E-cadherin, Snail1, Slug), RNPs, and HOXA genes 	[121, 126, 127]
HULC	6p24.3	500 bp	Upregulated	<ul style="list-style-type: none"> • Oncogenic • Promotes cell proliferation and invasion 	<ul style="list-style-type: none"> • Sponge for miR-200a-3p, miR-9, miR107 	[135, 136]
loc285194	3q13.31	2105 bp	Downregulated	<ul style="list-style-type: none"> • Tumor suppressive • Loss leads to osteoblast proliferation 	<ul style="list-style-type: none"> • Regulation of cell cycle and cell death genes • Regulation of VEGF1 transcription • Regulated by p53 • Represses miR-211 	[171, 173, 174]

lncRNA	Chr. locus	Transcript length	Expression	Function	Mechanisms	Refs
lncRNA-ATB	14q11.2	2.4 kb	Upregulated	<ul style="list-style-type: none"> Promotes cell proliferation, invasion, and metastasis 	<ul style="list-style-type: none"> Activated by TGFβ Enhances EMT Inhibits miR-200s Upregulates ZEB1/ZEB2–miR200s target genes 	[208, 209, 211, 212]
MALAT-1 (NEAT-2)	11q13.1	8.7 kb	Upregulated	<ul style="list-style-type: none"> Oncogenic Promotes cell proliferation, invasion, and metastasis Inhibits apoptosis 	<ul style="list-style-type: none"> Acts through PI3K/Akt and RhoA/ROCK pathways Competes with miR-376a Promotes TGFα upregulation Regulated by Myc-6 Upregulates MMP-9 and HDAC4 Decoy for miR-140-5p 	[87, 143, 144, 199]
MF12	3q29	951 bp	Upregulated	<ul style="list-style-type: none"> Oncogenic Promotes cell proliferation and migration 	<ul style="list-style-type: none"> Enhances FOXP4 expression 	[166]
MEG3	14q32.3	1.6 kb	Downregulated	<ul style="list-style-type: none"> Tumor suppressor 	<ul style="list-style-type: none"> Implicated in Wnt/β-catenin pathway Regulated by lncRNA EWSAT1 	[186, 221]
NKILA		2.5 kb	Downregulated	<ul style="list-style-type: none"> Promotes invasion and metastasis 	<ul style="list-style-type: none"> Regulates NF-κB activity through interaction with IκBα 	[214]
ODRUL	16q24.1	319 bp	Upregulated	<ul style="list-style-type: none"> Promotes invasion and metastasis 	<ul style="list-style-type: none"> Competes with miR-3182 Upregulates MMP2 	[217]
PACER	1q31.1	793 bp	Upregulated	<ul style="list-style-type: none"> Promotes cell proliferation, invasion, and metastasis 	<ul style="list-style-type: none"> NF-κB-dependent upregulation of COX-2 Regulated by CTCF 	[89]
SNHG12	1p35.3	1.3 kb	Upregulated	<ul style="list-style-type: none"> Oncogenic Promotes cell proliferation, invasion, and metastasis 	<ul style="list-style-type: none"> Increases angiomin expression Upregulation of Notch2 Sponge for miR-195-p2 Upregulation of MMP-2 and MMP-9 	[167, 168]
TUG1	22q12.2	7.1 kb	Upregulated	<ul style="list-style-type: none"> Oncogenic Promotes cell proliferation and invasion 	<ul style="list-style-type: none"> Interacts with PRC2 Sponge for miR-9-5p Decreases POUF2F1 expression EZH2 upregulation via miR-144-3p Inhibition of miR-212-3p 	[148–150]

lncRNA	Chr. locus	Transcript length	Expression	Function	Mechanisms	Refs
TUSC7	3q13.31	2 kb	Downregulated	<ul style="list-style-type: none"> • Tumor suppressive 	<ul style="list-style-type: none"> • Affects proapoptotic proteins expression 	[189, 190]
UCA1	19p13	2314 bp	Upregulated	<ul style="list-style-type: none"> • Oncogenic • Promotes cell proliferation • Inhibits cell death • Promotes invasion and metastasis 	<ul style="list-style-type: none"> • Interacts with CREB, BRG1, miR-216b, hnRNP1 • Involvement in PTEN/Akt/Bax/Bcl-2 pathway • Involvement in miR-216b/FGFR1/ERK pathway 	[157–161]
ZEB-AS1	2.6 kb	10p11.22	Upregulated	<ul style="list-style-type: none"> • Oncogenic • Promotes cell proliferation, invasion, and metastasis 	<ul style="list-style-type: none"> • Epigenetic regulation of ZEB1 transcription • Sponge for miR-200s 	[169, 170]

Table 1.
Expression, function, and mechanisms of lncRNAs in osteosarcoma.

H19 antisense RNA (91H) has a transcript length of 2.3 kb and is transcribed from the H19/IGF2 genomic imprinted cluster, and its gene is located on chromosome 11p15.5 [93]. It is involved in insulin-like growth factor 2 (IGF2) transcriptional regulation [94, 95]. It has also been observed that the IGF2 and H19 genes are imprinted in the majority of normal human tissues and IGF2 transcriptional repression is regulated through CTCF binding to the H19 imprinting control region [96]. On the other hand, imprinting is lost in various tumor types. Osteosarcoma specimens show maintenance or loss of IGF2/H19 imprinting depending on allele-specific differential methylation of the CTCF-binding regulatory site upstream of H19 gene [97]. Loss of imprinting of IGF2 or H19 in osteosarcoma is mutually exclusive [97]. H19 antisense RNA expression has been found to be elevated in osteosarcoma clinical specimens and osteosarcoma cell line and was correlated with advanced clinical stage. It was considered an independent prognostic factor for overall survival in treated osteosarcoma patients [98]. Moreover, H19 antisense RNA knockdown led to cell death promotion and inhibition of osteosarcoma proliferation, the mechanism of which needs to be elucidated [98].

Antidifferentiation noncoding RNA (ANCR), also called DANCR, is a lncRNA that has been found to suppress osteoblastogenesis through inhibition of Runx2 expression. ANCR interacts with the enhancer of zeste homolog 2 (EZH2). This interaction leads to H3K27me3 catalysis in Runx2 promoter resulting in inhibition of Runx2 expression and suppression of osteogenic differentiation [99]. ANCR also controls the cell cycle progression of osteosarcoma cells through regulation of expression levels of p21, CDK2, and CDK4 and other cell cycle-related proteins as well [100, 101].

Breast cancer antiestrogen resistance 4 (BCAR4) is another lncRNA that has been found to be involved in antiestrogen resistance in breast cancer cell lines [102, 103]. It also promotes cell growth and proliferation as well as invasion and metastasis in breast cancer cell lines, via the noncanonical Hedgehog/GLI2 pathway [75, 98]. In osteosarcoma, BCAR4 exerts its oncogenic action by activating GLI2-dependent gene transcription via direct promoter binding [104]. Upregulation of BCAR4 has been observed in osteosarcoma pathological specimens and is correlated with poor overall survival. Knockdown BCAR4 experiments have shown that suppression of BCAR4 inhibits proliferation and migration in vitro and in vivo through GLI2 target genes [105].

Fibroblast growth factor receptor 3 antisense transcript 1 (FGFR3-AS1), previously known as lncRNA-BX537709, is complementary to FGFR3 in an antisense direction and increases the mRNA stability and expression of FGFR3 through antisense pairing with the FGFR3 3'UTR [106]. FGFR3-AS1 is upregulated in osteosarcoma along with FGFR3 and is correlated with poor clinical outcome. Knockdown FGFR3-AS1 experiments in osteosarcoma cell lines have demonstrated that suppression of FGFR3-AS1 function leads to inhibition of cell cycle progression and cell proliferation [107].

HIF-2 α promoter upstream transcript (HIF2PUT), also named as TCONS_00004241, is located on chromosome 2p21 [80, 108]. It belongs to the class of promoter upstream transcripts lncRNAs (PROMPTs) which regulate host gene transcription [109–111]. In knockdown experiments, suppression of HIF2PUT led to inhibition of expression of HIF-2 α and stemness-related genes such as Oct4, Sox, and CD44, resulting in inhibition of cancer stem-cell properties [112]. In osteosarcoma, HIF-2 α mRNA and HIF2PUT expression levels are increased and are correlated with advanced clinical stage and poor disease-free and overall survival [80, 108]. HIF2PUT action in osteosarcoma tumorigenesis needs further elucidation in order to understand better its role in osteosarcoma cell self-renewal and stemness.

HOX transcript antisense RNA (HOTAIR) is a 2337-bp-long lncRNA with high expression levels in osteosarcoma tissue clinical specimens [113]. It is implicated in the pathogenesis of various tumors including hepatocellular carcinoma, lung carcinoma, and breast and ovarian cancers [114–117]. It promotes tumor cell growth and proliferation by inhibiting gene expression through histone H3K27 trimethylation, functioning as a modular scaffold by binding PRC2 through the 5' domain and LSD1/CoREST/REST complexes through the 3' domain [118, 119]. This molecular mechanism is implicated in other cancer types but remains to be elucidated in osteosarcoma. Interestingly, a genetic variant of HOTAIR, rs7958904, is associated with decreased risk of osteosarcoma in a two-stage case-control study in Chinese population with 900 osteosarcoma cases and 900 controls [120].

HOXA transcript at the distal tip (HOTTIP) is a lncRNA which is overexpressed in osteosarcoma specimens and is correlated with advanced clinical stage and high metastatic potential [121]. Elevated expression of HOTTIP is associated with increased tumor cell proliferation, migration, and invasion in a variety of malignant tumors [122–125]. It exerts its action through regulation of (i) EMT-related molecules such as E-cadherin, Snail1, Slug, etc., (ii) RNA-binding proteins, and (iii) HOXA genes such as HOXA13 [126, 127]. HOTTIP knockdown inhibits cell proliferation, migration, and invasion in osteosarcoma cell lines [42, 128].

Highly upregulated in liver cancer lncRNA (HULC) was initially identified to be upregulated in human hepatocellular carcinoma which has an oncogenic function. Its gene is located on chromosomal locus 6p24.3, has a transcript length of 500 bp, and associates with ribosomes [129, 130]. HULC acts as a sponge for different miRNAs, such as miR200a-3p, miR-9, and miR107, by reducing their expression [131, 132]. It promotes tumor cell growth, invasion, and angiogenesis in hepatocellular and colorectal carcinoma cell lines [133, 134]. HULC is overexpressed in osteosarcoma cell lines and tissue specimens, and its overexpression is correlated with advanced clinical stage and poor overall survival in osteosarcoma patients. HULC inhibition reduces cell proliferation and invasion in osteosarcoma cell lines [135, 136].

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT-1), also called noncoding nuclear-enriched abundant transcrip. 2 (NEAT-2), has a 8.7-kb transcript, and its chromosomal locus is on 11q13 [137]. It is a nuclear lncRNA, initially found to be upregulated in non-small cell lung adenocarcinoma [137, 138]. MALAT-1 functions as a competitive endogenous RNA (ceRNA) by binding to different miRNAs that regulate the transcription of genes such as cell division cycle 2 (cdc2) through miR-1 in breast carcinoma cells [139], Slug through miR-204 in lung adenocarcinoma [140] and metalloproteinase-14 (MMP14), and Snail through miR-22 in melanoma [141]. MALAT-1 is highly expressed in osteosarcoma tissue samples and is correlated with metastatic dissemination and advanced clinical stage [142, 143]. MALAT-1 acts through the PI3K/Akt pathway to promote osteosarcoma cell proliferation, migration, invasion, and pulmonary metastasis [87]. Furthermore, MALAT-1 inhibition suppressed osteosarcoma cell proliferation and metastasis via the PI3K/Akt and RhoA/ROCK signaling pathway by decreasing the expression levels of proliferating cell nuclear antigen (PCNA), Act and phosphorylated PI3Kp85 α , as well as MMP-9 metalloproteinase, as mentioned in the signal transduction section [87, 88]. In addition, MALAT-1 may contribute to osteosarcoma tumorigenesis and progression by competing miR376A and promotes TGF α upregulation [144]. MALAT-1 downregulation is also involved in Myc-6 osteosarcoma suppressor activity in MG63 osteosarcoma cell line [145].

Taurine upregulated gene 1 (TUG1) is a 7.1-kb lncRNA, and its gene is located on chromosomal locus 22q12.2 [146]. It seems to be induced by p53, interacts with

polycomb repressive complex 2 (PRC2), and suppresses specific genes involved in the G0/G1 cell cycle arrest, facilitating osteosarcoma tumorigenesis [147]. In this context, TUG1 acts as a sponge for miR-9-5p and decreases the expression of POU class 2 homeobox 1 (POUF2F1) supporting the presence of a competitive miR-lncRNA regulatory network [148]. It also promotes osteosarcoma tumorigenesis through EZH2 upregulation via miR-144-3p [149]. Additionally, TUG1 knockdown represses the activation of Wnt/ β -catenin pathway, which is reversed by EZH2 upregulation [149]. TUG1 is also involved in osteosarcoma cell proliferation and invasion through inhibition of miR-212-3p [150]. Interestingly, osteosarcoma tissue clinical samples exhibit high expression levels of TUG1, and impairment of TUG1 expression in osteosarcoma cell line U2OS inhibits cell proliferation and promotes cell death [151]. TUG1 is overexpressed in osteosarcoma tissue specimens, and its overexpression is associated with unfavorable prognosis [152].

Urothelial carcinoma associated 1 (UCA1) is a 2314-bp lncRNA located on chromosome 19 and initially identified in bladder carcinoma [153]. It is upregulated in many different tumor types including osteosarcoma, and its overexpression is correlated with high tumor grade, distant metastatic dissemination, advanced clinical stage, and poor clinical outcome [154–156]. Overexpression of UCA1 promotes cancer cell proliferation through interactions with CREB, BRG1, miR-216b, or hnRNP1 [158–161]. On the other hand, UCA1 overexpression inhibits cell death through Akt/Bax/Bcl-2 signaling pathway and promotes migration, invasion, and metastasis via the miR-216b/FGFR1/ERK signal transduction pathway [157–160]. UCA1 upregulation has also been found to be implicated in increased drug resistance through SPRK1, Wnt6, and Wnt signaling pathways [162–164]. UCA1 knockdown experiments in osteosarcoma cell lines have shown that suppression of UCA1 function leads to promotion of cell death and inhibition of cell cycle progression, cell proliferation, cell migration, and invasion, whereas UCA1 upregulation displays opposite effects [160, 165].

Other lncRNAs that play important role in osteosarcoma cell proliferation and display oncogenic properties are:

Modified frailty index 2 (MFI2) is implicated in osteosarcoma development and proliferation by enhancing forkhead box P4 (FOXP4) expression [166].

Small nucleolar RNA host gene 12 (SNHG12) acts by increasing expression of angiomin gene in human osteosarcoma cell lines and through this action regulates cell proliferation [167]. SNHG12 is also involved in the promotion of osteosarcomagenesis and metastasis through upregulation of Notch2, acting as a sponge for miR-195-p2 in 143B and U2OS osteosarcoma cells [168].

ZEB1 Antisense 1 (ZEB1-AS1) is upregulated in osteosarcoma and promotes osteosarcoma cell proliferation via epigenetic regulation of ZEB1 transcription [169]. ZEB1-AS1 also acts as a sponge for miR-200s and through this action reverses the ZEB1 inhibition caused by miR-200s [170].

3.2 Tumor suppression lncRNAs

Another lncRNA category that plays a significant role in osteosarcoma tumorigenesis includes lncRNAs with tumor suppressive properties such as Loc285194, MEG3, and TUSC7. These lncRNAs are summarized in **Table 1** along with their function and mechanisms.

Loc285194, also named **LSAMP antisense RNA3**, is a 2105-bp lncRNA encoded on chromosomal locus 3q13.31, also called as osteo3q13.31, a locus with frequent copy number alterations and loss of heterozygosity in osteosarcoma [171, 172]. Loc285194 is downregulated in osteosarcoma cell lines and tissue specimens. Loc285194 loss leads to increased osteoblast proliferation through regulation of cell

cycle and cell death-related transcripts. It is also implicated in the regulation of VEGF1 transcript [171]. Studies on HCT-116 colon cancer cell line have shown that Loc285194 transcription is regulated by p53 [173, 174] and acts as a tumor suppressor by direct repression of miR-211 in a reciprocal negative feedback loop [175]. This mechanism has not yet been established in osteosarcoma cell lines.

Maternally expressed gene 3 (MEG3) is a lncRNA transcribed by an imprinted gene located on the chromosome 14q32.3 DLK1-MEG3 locus [176]. Reduced or loss of MEG3 expression has been found in many different tumor types such as non-small lung cancer, gastric cancer, colorectal cancer and bladder cancer [177]. The underlying mechanism is through epigenetic promoter or intergenic hypermethylation [178]. Induced expression of MEG3 in different cancer cell lines leads to inhibition of cell proliferation, suppression of migration and invasion, and promotion of apoptosis as well [179–182]. MEG3 overexpression also reduced the expression level of miR21-5p in cervical cancer cells [183], increased the levels of p53, and stimulated the transcription of p53-dependent genes such as MDM2 [184]. It is also implicated in the Wnt/ β -catenin signaling pathway through regulation of p53, β -catenin, and survivin [185, 186]. Osteosarcoma tissue samples display reduced MEG3 expression levels, and its low expression is associated with distant metastatic dissemination [187, 188]. Further studies are needed to confirm the role of MEG3 in osteosarcoma pathogenesis.

Tumor Suppressor Candidate 7 (TUSC7) is a lncRNA which is downregulated in osteosarcoma cell lines resulting in cell proliferation promotion and increased colony formation in vitro. Decreased expression levels in osteosarcoma tissue specimens are associated with poor survival in osteosarcoma patients. TUSC7 silencing in HOS and MG63 osteosarcoma cells affects the expression of proapoptotic proteins resulting in decreased levels, but with no effect on cell cycle regulation. Moreover MG63 xenografts in nude mice showed tumor growth in vivo after TUSC7 silencing [189, 190].

4. LncRNAs and cell death in osteosarcoma

It is well known that tumor cells enhance their viability by inhibiting apoptosis and anoikis and can survive and metastasize in distant body sites and diverse microenvironments. By inhibiting or reducing the activity of cell death machinery, tumors become resistant to various therapeutic interventions and progress to advanced clinical stages [191–194]. Recent studies have demonstrated the involvement of lncRNAs in osteosarcoma cell death and make them putative therapeutic targets for more efficient osteosarcoma treatment [195, 196].

Reduced **91H** lncRNA expression levels promote osteosarcoma apoptosis via upregulation of miR-141. Overexpression of miR-141 in hFOB1.19 cells leads to osteosarcoma cell apoptosis through the suppression of H19 and miR-675 expression resulting in reduced Bcl-2/Bax ratio and caspase-3 expression [197]. Moreover, knockdown of H19 lncRNA leads to cell death promotion and inhibition of osteosarcoma proliferation.

Inhibition of **BRAF-activated noncoding RNA (BANCR)** lncRNA suppresses MG63 osteosarcoma cell proliferation and invasion in vitro and promotes cell death as well [198].

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) lncRNA affects the apoptotic osteosarcoma cell machinery through the RhoA/ROCK signal transduction pathway [88]. MALAT1 also regulates osteosarcoma cell proliferation and apoptosis through upregulation of histone deacetylase 4 (HDAC4) by decoying miR-140-5p [199].

Overexpression of **MF12** lncRNA suppresses osteosarcoma cell apoptosis through FOXP4 transcription regulation. Additionally, MF12 knockdown in MG-63 and Saos-2 osteosarcoma cell lines induces apoptosis and reduces cell growth, migration and invasion. [166].

Taurine upregulated gene 1 (TUG1) lncRNA overexpression promotes osteosarcoma tumorigenesis by suppressing specific genes involved in the G0/G1 cell cycle arrest [147]. In this context, TUG1 acts as a sponge for miR-9-5p and decreases the expression of POU class 2 homeobox 1 (POUF2F1) [148]. Suppression of TUG1 has been demonstrated that inhibits cell proliferation and significantly promotes osteosarcoma apoptosis [151].

Silencing of tumor suppressor lncRNA **TUSC7** in HOS and MG63 osteosarcoma cells reduces the expression levels of proapoptotic proteins and results in apoptotic cell reduction [189, 190].

Further studies are needed to explore the role and the precise mechanisms of these lncRNAs in osteosarcoma cell death in an attempt to modulate their action for therapeutic reasons.

5. LncRNAs and invasion/metastasis in osteosarcoma

Despite the introduction of modern treatment approaches by applying multimodality therapies in osteosarcoma patients and the improvement in disease-free survival, the overall long-term survival remains relatively low. In patients with localized disease, the 5-year relapse-free survival is around 75–80% for the good chemoresponders, compared with 45–55% for the poor chemoresponders, in the adjuvant setting and after surgical removal of the bone tumor. The rest of the patients will display mainly pulmonary metastasis by relapsing within the first 5 years, probably because of the presence of undetectable metastatic disease at the time of the initial diagnosis. Approximately, 20–25% of newly diagnosed osteosarcoma patients are presenting with metastatic disease at the initial diagnosis. These patients have an unfavorable prognosis with overall survival rates around 10–30%. It is obvious that the main cause of the high mortality seen in those patients is the development of metastasis, mainly in the lungs [12–18]. Thus, it is important, in order to improve the outcome of patients with metastatic disease, to get insight into the underlying mechanism of osteosarcoma metastasis and develop new therapeutic agents against the metastasis regulatory pathways.

The metastatic process may occur through three main pathways in general: (1) direct invasion of adjacent organs or seeding of body cavities, (2) lymphatic spread, and (3) hematogenous spread. The latter is the main pathway of osteosarcoma metastatic dissemination. A major role in the metastatic cascade plays the phenomenon of epithelial to mesenchymal transition (EMT) whereby epithelial cells lose their epithelial features and acquire mesenchymal cells traits which allow them to invade adjacent tissues and display migratory properties. The metastatic cascade is a multistep complex process and can be divided in the following phases: (1) invasion of the extracellular matrix (ECM) and degradation of ECM proteins through the activity of matrix metalloproteinases (MMPs), (2) intravasation, (3) resistance to anoikis and survival in the peripheral blood, (4) extravasation, and (5) seeding of a distant body site by clones of neoplastic cells with high metastatic potential [192, 200, 201]. A number of studies have shown the involvement of lncRNAs in the metastatic progression of osteosarcoma through modulation of metalloproteinase expression, especially MMP-2 and MMP-9 [202, 203].

Breast cancer antiestrogen resistance 4 (BCAR4) is a lncRNA whose expression has been found to be increased in osteosarcoma tissue specimen in patients

with lung metastasis [204]. It acts through transcriptional activation of GLI2-dependent genes via direct promoter binding. Suppression of BCAR4 leads to inhibition of proliferation and migration of osteosarcoma cells in vitro and in vivo through GLI2 target genes [104, 205].

Differentiation antagonizing non-protein coding RNA (DANCR), also named ANCR, is a lncRNA that has been found to be overexpressed in osteosarcoma tissue specimens and in osteosarcoma cell lines. It is involved in osteosarcoma cell proliferation and metastasis through Rho-associated coiled-coil-containing protein kinase 1 (ROCK1) mediation via decoying both miR-335-5p and miR-1972 microRNAs. In this context DANCR acts as a metastasis-promoting lncRNA by playing the role of a competing endogenous RNA (ceRNA) [206].

HOX transcript antisense RNA (HOTAIR) is highly expressed in osteosarcoma and is correlated with distant metastasis and advanced clinical stages. It promotes osteosarcoma invasion through upregulation of metalloproteinases MMP-2 and MMP-9 [207].

Highly up-regulated in liver cancer lncRNA (HULC) acts as a sponge for different miRNAs, such as miR200a-2p, miR-9, and miR107, by reducing their expression [131, 132]. HULC is overexpressed in osteosarcoma cell lines and tissue specimens, and its overexpression is correlated with distant metastasis, advanced clinical stage, and poor overall survival in osteosarcoma patients [135]. It promotes tumor cell growth, invasion, and angiogenesis in different cell lines, and its inhibition reduces cell proliferation and invasion in osteosarcoma cell lines [136].

Long noncoding RNA activated by transforming growth factor- β (lncRNA-ATB) is a novel lncRNA which is activated by the TGF- β and plays a crucial role in many cancers [208]. EMT, and thus invasiveness, can be enhanced by the involvement of the lncRNA-ATB, which acts by interfering the action of miR-200s, a microRNA that suppresses ZEB1 and ZEB2 action [209]. LncRNA-ATB expression levels are high in hepatocellular carcinoma as compared to normal liver samples and are correlated with vascular invasion [210]. Moreover, orthotopic mice injected by hepatocellular carcinoma cells overexpressing lncRNA-ATB developed distant metastasis [211]. LncRNA-ATB promotes osteosarcoma cell proliferation, migration and invasion by inhibiting miR-200s and upregulating the ZEB1 and ZEB2 miR-200s target genes. LncRNA-ATB is also overexpressed in osteosarcoma tissue samples and cell lines and positively correlated with advanced clinical stage, metastasis, and recurrence [212]. The role of lncRNA-ATB in osteosarcoma metastasis is not yet well established, and more studies need to be done in order to elucidate its involvement.

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT-1) facilitates osteosarcoma invasion and metastasis by suppressing the microRNA 376A (miR376A) and promoting TGF α upregulation [144]. MALAT-1 also acts through the PI3K/Akt pathway to promote osteosarcoma cell proliferation, migration, invasion, and pulmonary metastasis. Furthermore, MALAT-1 knockdown or siRNA interference experiments, carried out by Dong et al. and Cai et al., respectively, showed that MALAT1 inhibition suppressed osteosarcoma cell proliferation and metastasis via the PI3K/Akt and RhoA/ROCK signaling pathways by modulating the expression of MMP-9 metalloproteinase [85, 87, 88]. MALAT-1 is highly expressed in osteosarcoma tissue samples and is correlated with metastatic dissemination and advanced clinical stage [142, 143].

Nuclear factor – κ B interacting lncRNA (NKILA) is a 2.5-kb lncRNA that negatively regulates the NF- κ B pathway. NF- κ B is a transcription factor that mediates inflammatory signal transduction processes [213]. It is constitutively active in various tumor types, and its activity can be modulated by interacting with NKILA (nuclear factor- κ B interacting lncRNA). NKILA regulates NF- κ B activity via

interaction with $\text{I}\kappa\text{B}\alpha$, a negative regulator of NF- κB translocation from the cytoplasm to the nucleus, thus preventing the transcriptional activation of NF- κB dependent genes [214]. Loss or low expression of NKILA is correlated with advanced clinical stage and metastatic dissemination in breast cancer patients [215]. The role of NKILA in osteosarcoma metastatic dissemination is not well known and remains to be confirmed.

Osteosarcoma doxorubicin resistance-related up-regulated lncRNA (ODRUL) expression levels have been found to be elevated in osteosarcoma tissue specimens of patients with pulmonary metastasis [216]. ODRUL upregulates MMP2 expression through direct competing interaction with miR-3182 and thus promotes invasion and metastasis [217]. ODRUL knockdown experiments in osteosarcoma cell lines led to inhibition of tumor proliferation and invasion by decreasing matrix metalloproteinase (MMP) expression, showing an important role in osteosarcoma metastatic process [217].

P50-associated COX-2 extragenic RNA (PACER) is another lncRNA that acts by promoting osteosarcoma invasion and metastasis through NF- κB -dependent upregulation of COX-2 gene [89].

Small nuclear RNA host gene 12 (SNHG12) lncRNA has been demonstrated to be implicated in the induction of osteosarcoma cell proliferation and migration through the angiominin upregulation which in turn controls the expression levels of MMP-2 and MMP-9 [167]. SNHG12 is also involved in the promotion of osteosarcomagenesis and metastasis through upregulation of Notch2, acting as a sponge for miR-195-p2 in 143B and U2OS osteosarcoma cells [168].

Zinc finger E-box binding homeobox 1 Antisense 1 (ZEB1-AS1) has been found to display elevated expression levels in metastatic osteosarcoma and regulate the metastatic process by increasing ZEB1 transcription [169]. ZEB1, in turn, promotes invasion and metastasis by inducing epithelial-mesenchymal transition (EMT). ZEB1-AS1 also acts as a sponge for miR-200s and through this action reverses the ZEB1 inhibition caused by miR-200s [170].

Other lncRNAs that play an important role in osteosarcoma invasion and metastasis are:

lncRNA MF12 that has been shown to promote migration of osteosarcoma cells via FOXP4 upregulation [166]. In addition, overexpression of **urothelial carcinoma associated 1 (UCA1)** and **BRAF-activated noncoding RNA (BANCR)** lncRNAs is correlated with metastasis in distant body sites [165, 198].

All the abovementioned lncRNAs are summarized in **Table 1** along with their function and mechanisms.

Further unraveling the mechanism of osteosarcoma invasiveness and metastatic dissemination and the possible involvement of lncRNAs in this process will provide useful insights to develop new therapeutic targets for the management of metastatic osteosarcoma and improve the long-term survival of patients.

6. lncRNAs as prognostic biomarkers in osteosarcoma

The efficacy of osteosarcoma treatment and the accurate prognosis of the clinical outcome depend on clinical, histopathological, and molecular factors, and therefore, it is important to identify and incorporate prognostic factors into a holistic therapeutic strategy. Age, gender, anatomic location, tumor size, and a variety of biological molecules have been used and proposed as a tool to predict the treatment responsiveness and the clinical outcome/prognosis. Recent studies have indicated that lncRNAs may be of clinical value and may be used as prognostic biomarkers in osteosarcoma [106, 128, 218].

Upregulation of **fibroblast growth factor receptor 3 antisense transcript 1 (FGFR3-AS1)** lncRNA is correlated with advanced Enneking surgical stage, large tumor size, and poor clinical outcome and survival [107]. Based on these observations, its expression levels could serve as a prognostic factor.

Interestingly, in a two-stage case-control study in Chinese population performed by Zhou et al., they found that a genetic variant of **HOTAIR**, rs7958904 the CC genotype, was associated with decreased risk of osteosarcoma compared with the G allele (OR, 0.77; 95% CI, 0.67–0.90; $P = 6.77 \times 10^{-4}$). About 900 osteosarcoma patients and 900 control subjects have been evaluated, and the findings suggested that HOTAIR rs7958904 CC genotype patients had significant lower HOTAIR expression levels compared to other genotype patients, as well as lower osteosarcoma risk. Therefore HOTAIR can be used as a prognostic factor for osteosarcoma risk assessment [120].

HOXA transcript at the distal tip (HOTTIP) lncRNA overexpression in osteosarcoma human tissue specimens is associated with distant metastasis, advanced clinical stage, and unfavorable prognosis. Elevated HOTTIP expression levels have been demonstrated to correlate with poor overall survival and to be an independent prognostic factor [121].

Highly upregulated in liver cancer (HULC) lncRNA is overexpressed in osteosarcoma cell lines and tissue specimens, and its overexpression is correlated with advanced clinical stage and poor overall survival in osteosarcoma patients [135, 136]. HULC acts as a sponge for different miRNAs, such as miR200a-2p, miR-9, and miR107, by reducing their expression, and leads to increased cell proliferation, cell migration, and invasion in osteosarcoma cell lines [131, 132, 134, 205]. Inactivation of HULC via knockdown experiments and/or upregulation of miR-122 via transfection of osteosarcoma cell lines results in inactivation of PI3K/Act, Notch, and Jak/Stat pathways leading in reduced proliferation, migration, and invasion [219]. Therefore, HULC could be used as a prognostic factor for osteosarcoma patients as high expression levels are positively correlated with distant metastasis and advanced clinical stage.

Activated by transforming growth factor beta (lncRNA-ATB) displays high expression levels in osteosarcoma cell lines and tissues. Patients with osteosarcoma have elevated serum expression levels of lncRNA-ATB, and this overexpression is correlated with local recurrence, distant metastasis, and advanced clinical stage [208, 212]. Thus, lncRNA-ATB could be used as a prognostic and recurrence monitoring factor for osteosarcoma patients.

Maternally expressed gene 3 (MEG3) lncRNA expression levels are decreased in osteosarcoma tissues compared with adjacent normal tissues and are associated with distant metastasis, advanced clinical stage, and poor overall survival [177, 220]. Its expression is regulated by lncRNA Ewing sarcoma associated transcript 1 (EWSAT1) and downregulation of MEG3 in the presence of EWSAT1 induces osteosarcoma cell proliferation, invasion, and metastasis [221]. Therefore, high levels of MEG3 could be an indicator of favorable prognosis in osteosarcoma patients.

Taurine upregulated gene 1 (TUG1) lncRNA has been found to be overexpressed in osteosarcoma human samples compared with normal matched tissues ($P < 0.01$), and expression levels were associated with tumor size, postoperative chemotherapy responsiveness and Enneking surgical stage [152]. Moreover, TUG1 high expression levels were significantly correlated with unfavorable prognosis and were an independent prognostic factor for disease-free survival (HR = 1.81; 95% CI = 1.01–3.54; $P = 0.037$) and long-term overall survival (HR = 2.78; 95% CI = 1.29–6.00; $P = 0.009$). Interestingly, TUG1 elevated plasma levels are associated with disease progression or relapse [152]. Thus, TUG1 might be used as a prognostic and monitoring biomarker for osteosarcoma patients.

lncRNA	Expression in osteosarcoma	Clinical value	Role in drug resistance or sensitivity	Mechanism of drug resistance or sensitivity	Therapeutic target	Agent targeting lncRNA	Refs
BCAR4	Upregulated	Prognostic, therapeutic			Yes	Antagonist	[105, 204, 205]
BANCR	Upregulated	Prognostic, predictive	Adriamycin resistance	—			[198]
CASC2	Downregulated	Prognostic, therapeutic			Yes	Agonist-mimic	[242]
FENDRR	Downregulated	Predictive, therapeutic	Doxorubicin resistance	<ul style="list-style-type: none"> Upregulated acts as a suppressor of doxorubicin resistance by inhibiting ABCB1 and ABCC1 expression 	Yes	Agonist-mimic	[229]
FGFR3-AS1	Upregulated	Prognostic					[107]
FOXC2-AS1	Upregulated	Prognostic, predictive	Doxorubicin resistance	Induces ABCB1 gene expression			[230]
GAS5	Downregulated	Prognostic, therapeutic			Yes	Agonist-mimic	[243, 244]
HOTAIR	Upregulated	Prognostic, risk assessment					[120, 207]
HOTTIP	Upregulated	Prognostic, predictive	Cisplatin resistance	<ul style="list-style-type: none"> Activates Wnt/β-catenin pathway 			[231]
HULC	Upregulated	Prognostic					[135, 136]
LINC00161	Upregulated	Predictive	Cisplatin sensitivity	<ul style="list-style-type: none"> Promotes apoptosis Increases IFIT2 expression Sponge for miR-645 			[232]
LncRNA-ATB	Upregulated	Prognostic, monitoring marker					[212]
LUCAT1	Upregulated	Predictive	Methotrexate resistance	<ul style="list-style-type: none"> Interacts with ABCB1 through miR-200c Regulates miR-200c expression 			[235]

lncRNA	Expression in osteosarcoma	Clinical value	Role in drug resistance or sensitivity	Mechanism of drug resistance or sensitivity	Therapeutic target	Agent targeting lncRNA	Refs
MALAT-1	Upregulated	Prognostic, therapeutic			Yes	Antagonist	[142, 143, 246]
MEG3	Downregulated	Prognostic					[188, 220]
MF12	Upregulated	Prognostic, therapeutic			Yes	Antagonist	[166]
NBAT1	Downregulated	Prognostic, therapeutic			Yes	Agonist-mimic	[248]
NR-036444	Upregulated	Predictive	Doxorubicin resistance	Interacts with ABCB1, HIF1 α , and FOXC2			[236]
ODRUL	Upregulated	Prognostic, predictive	Doxorubicin resistance	Induces ABCB1 gene expression			[217, 237]
PANDA	Upregulated	Prognostic, therapeutic	Doxorubicin resistance	<ul style="list-style-type: none"> Increased expression after doxorubicin and etoposide treatment Depletion promotes apoptosis through upregulation of APAF1, BIK, FAS, and LRDD 	Yes	Antagonist	[249, 250]
PVT1	Upregulated	Prognostic, therapeutic			Yes	Antagonist	[251, 252]
TP73-AS1	Upregulated	Prognostic, therapeutic			Yes	Antagonist	[253, 254]
TUG1	Upregulated	Prognostic, monitoring marker					[152]
ZEB1-AS1	Upregulated	Prognostic					[169]

Table 2.
Potential clinical value of lncRNAs in osteosarcoma.

Zinc finger E-box binding homeobox 1 antisense 1 (ZEB1-AS1) has been found to display elevated expression levels in metastatic osteosarcoma and regulate the metastatic process by increasing ZEB1 transcription [169, 170]. Overexpression of ZEB1-AS1 is associated with advanced clinical stage, large tumor size, distant metastatic dissemination, and unfavorable progression-free and overall survival [169]. In clinical setting, ZEB1-AS1 could serve as a prognostic marker for osteosarcoma patients.

All the abovementioned lncRNAs are summarized in **Table 2**.

7. LncRNAs as predictive biomarkers and drug resistance in osteosarcoma

A number of research teams have demonstrated the involvement of lncRNAs in chemoresistance and chemosensitivity of different types of cancer [222–227]. In osteosarcoma, chemotherapy plays an important role, but its efficacy is limited by acquired resistance to different chemotherapeutic drugs, mainly cisplatin and doxorubicin [228]. Recent studies have revealed the role of several lncRNAs that are related to osteosarcoma drug resistance such as FENDRR, ENST00000563280, HOTTIP, LINC00161, LUCAT1, NR-036444, and ODRUL [106].

FENDRR is another lncRNA which is significantly downregulated in doxorubicin-resistant osteosarcoma cell lines compared with the doxorubicin-sensitive counterparts (MG63/DXR vs. MG63, KH-OS/DXR vs. KH-OS, and U2-OS/DXR vs. U2-OS). In a microarray study FENDRR displayed a 22-fold decrease of its expression in doxorubicin-resistant MG63/DXR cells relative to their parental cell line MG63. It has been demonstrated that it acts as a suppressor of doxorubicin drug resistance by inhibiting ABCB1 and ABCC1 expressions [229].

Another lncRNA related with doxorubicin resistance in osteosarcoma cell lines is **forkhead box protein C2 antisense 1 (FOXC2-AS1)** also known as **ENST00000563280**. FOXC2-AS1 has been found to have elevated expression levels in osteosarcoma tissues and osteosarcoma cell lines resistant to doxorubicin, such as MG-63 and KH-OS. FOXC2-AS1 overexpression is associated with unfavorable clinical outcome and promotion of doxorubicin resistance in cell cultures. FOXC2-AS1 knockdown reversed the doxorubicin resistant phenotype and increased the doxorubicin sensitivity in osteosarcoma cells resistant to doxorubicin [230]. In addition, FOXC2 is overexpressed in osteosarcoma doxorubicin-resistant human tissues and cell lines, such as MG63/DXR and KH-OS/DXR, and its levels show positive correlation with FOXC2-AS1 expression. Both FOXC2-AS1 and FOXC2 are involved in doxorubicin resistance by inducing the expression of ABCB1 multidrug resistance gene [230]. Therefore, FOXC2-AS1 might serve as a predictive factor for doxorubicin sensitivity or resistance in osteosarcoma patients.

HOTTIP lncRNA is overexpressed in osteosarcoma specimens and is correlated with advanced clinical stage and high metastatic potential [121]. In a recent study, Li et al. found that overexpression of HOTTIP confers resistance to cisplatin in osteosarcoma cells in vitro through activation of the Wnt/ β -catenin pathway. Moreover, treatment with Wnt/ β -catenin inhibitor XAV939 or downregulation of HOTTIP reverses the cisplatin resistance [231]. Thus, HOTTIP expression levels might serve as a predictive biomarker regarding cisplatin resistance in osteosarcoma.

Long intergenic non-protein coding RNA 161 (LINC00161) is a lncRNA located on chromosome 21q21 locus and has been found to be overexpressed in cisplatin-treated osteosarcoma cells facilitating the cisplatin-induced apoptosis. Upregulation of LINC00161 in osteosarcoma cells promotes apoptosis by increasing

IFIT2 expression levels through the impairment of miR-645 action. In this context, LINC00161 acts as a sponge for miR-645, a microRNA that controls IFIT2 transcription [232].

Lung cancer associated transcript 1 (LUCAT1) lncRNA has been found to be overexpressed in osteosarcoma tissue samples and in MG63 and HOX osteosarcoma cell lines resistant to methotrexate, a drug that is used widely in osteosarcoma patients [233–235]. MG63 and HOX, resistant to methotrexate, also overexpress the ATP-binding cassette subfamily B member 1 (ABCB1), a drug resistance-related protein. LUCAT1 interacts with ABCB1 through miR-200c binding to the 3'UTR of ABCB1. Moreover, miR-200c expression is regulated in a LUCAT1-dependent manner. In addition, LUCAT1 knockdown experiments resulted in decreased expression levels of drug resistance-related genes MDR1, MRP5, and LRP1 in methotrexate-treated osteosarcoma cell lines and led to reduced osteosarcoma cell invasiveness [235]. Therefore, LUCAT1 expression levels might be used as a predictive biomarker providing information regarding methotrexate resistance or sensitivity.

NR-036444 is another lncRNA involved in an lncRNA-mRNA coexpression network and has been found to interact with doxorubicin-resistance related genes such as ABCB1, HIF1A, and FOXC2 in osteosarcoma cells and thus could serve as a predictive biomarker for chemoresistance [236].

Osteosarcoma doxorubicin resistance-related up-regulated lncRNA (ODRUL) has been initially found to be highly upregulated in the human osteosarcoma doxorubicin-resistant cell line MG63/DXR. Moreover ODRUL expression is elevated in human tissue osteosarcoma specimens from patients with poor response to doxorubicin therapy and lung metastasis. It has also been found that doxorubicin-sensitive osteosarcoma cell lines have reduced ODRUL expression levels. Additionally, ODRUL knockdown experiments in osteosarcoma cell lines led to inhibition of tumor proliferation and invasion and partly reversed the doxorubicin resistant phenotype through suppression of the multidrug resistance ABCB1 (ATP-binding cassette, subfamily B, member 1) gene [217, 237].

All the abovementioned lncRNAs are summarized in **Table 2**.

Further studies are needed to elucidate the role of lncRNAs in osteosarcoma drug resistance and exploit their potential as predictive biomarkers and candidates to develop novel therapeutic approaches in order to reverse the osteosarcoma resistance to chemotherapy.

8. lncRNAs as therapeutic targets in osteosarcoma

Treating osteosarcoma is a challenge in the practice of oncology. The main therapeutic approach is surgical removal of the tumor following by the application of chemotherapeutic agents such as doxorubicin, cisplatin, methotrexate in combination with leucovorin (folinic acid), and ifosfamide [13, 233]. This multimodal osteosarcoma management increased the progression-free survival rates from 10 to 20% up to 60% in recent years. Despite the relatively good cure rates of patients with localized tumor, unfortunately a percentage of 20–25% of newly diagnosed osteosarcoma patients are presenting with metastatic disease at the time of initial diagnosis. These patients have an unfavorable prognosis with overall survival rates around 10–30% [12–18]. In addition many patients develop resistance to available chemotherapeutic modalities and subsequently metastatic dissemination with unfavorable clinical outcome [228]. In recent years there are great efforts to exploit the molecular mechanisms of the metastatic process and drug resistance of osteosarcoma in order to develop novel therapeutic agents targeting biomolecules

involved in these processes. Such biomolecules, among others, are the lncRNAs which play important roles in the pathogenesis and progression of osteosarcoma [238–240].

Breast cancer antiestrogen resistance 4 (BCAR4) is another lncRNA that promotes cell growth and proliferation as well as invasion and metastasis in breast cancer cell lines cultures, via the noncanonical Hedgehog/GLI2 pathway [103, 104]. In osteosarcoma, BCAR4 exerts its oncogenic action by activating GLI2-dependent gene transcription via direct promoter binding [104]. Upregulation of BCAR4 has been observed in osteosarcoma pathological specimens and is correlated with advanced clinical stage, lung metastasis, and poor overall survival [105]. Knock-down BCAR4 experiments have shown that suppression of BCAR4 leads to inhibition of cell proliferation and migration in vitro and in vivo through downregulation of GLI2 target genes, such as IL-6, TGF-beta, RPS3, and MUC5AC [104]. Thus BCAR4 could be used as a target in osteosarcoma therapeutic management [205].

Cancer susceptibility candidate 2 (CASC2) was first discovered in patients with endometrial carcinoma as a potential tumor suppressor [241]. It is also significantly downregulated in osteosarcoma human specimens and various osteosarcoma cell lines such as MG-63, Saos-2, U2OS, and SOSP-9607, and its low expression levels correlate with poor survival and advanced clinical stage [241]. Interestingly, overexpression of CASC2 results in inhibition of osteosarcoma cell proliferation, colony formation, and invasion in vitro. Ectopic expression of CASC2 suppresses miR-181a expression and leads to upregulation of miR-181a target genes such as RASSF6, PTEN, and ATM in osteosarcoma cell lines. RASSF6 has been observed to positively correlate with CASC2 expression levels, and low RASSF6 levels have been found in osteosarcoma. In addition, in vivo implantation studies using pcDNA-CASC2 resulted in reduced tumor growth, while experiments using short interfering CASC2 exhibited enhanced tumor growth [242]. Consequently, CASC2 mimics might be of clinical value in osteosarcoma treatment in order to reduce tumor growth and slow down adverse clinical progression.

LncRNA growth arrest-specific 5 (GAS5) functions as an oncosuppressor lncRNA by repressing osteosarcoma cell proliferation and migration through sponging of miR-203a. In addition, silencing of lncRNA GAS5 significantly promotes osteosarcoma cell growth, migration, and invasion through upregulation of Cyclin D1, Cyclin B1, CDK1, and CDK4 expressions. Moreover, suppression of miR-203a leads to the reversion of GAS5 silencing effects [243]. GAS5 also functions as a ceRNA by binding to miR-221 resulting in the suppression of epithelial-mesenchymal transition and arrest of cell growth in osteosarcoma cell lines through regulation of the miR-221/ARHI axis [244]. Thus, GAS5 mimics could be used to slow down or suppress the osteosarcoma metastatic process.

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is an oncogenic lncRNA that is overexpressed in various osteosarcoma cell lines such as U2OS, Saos-2, and HOS and in human osteosarcoma tissue samples as well. Its overexpression is highly related to the metastatic potential of the tumor [142, 143]. MALAT1 acts through the PI3K/Akt and the RhoA/ROCK signaling pathway to promote osteosarcoma cell proliferation, migration, invasion, and pulmonary metastasis [87]. Downregulation of MALAT1 leads to reduced expression levels of RhoA and ROCK1 and 2 in osteosarcoma cell lines [87, 88]. Moreover, MALAT1 knockdown induces cell cycle arrest at the G0/G1 to S phase leading to reduced cell proliferation and invasion and enhanced apoptosis in HOS and U2OS cell lines. In addition, MALAT1 knockdown affects negatively the ability of osteosarcoma cells to form new blood circulatory networks in three-dimensional cell cultures [88, 245]. In addition, MALAT1 knockdown inactivates the Rac1/JNK signal transduction pathway through activation of miR-509 and downregulation of high mobility group

protein B1 (HMGB1) [246, 247]. It is obvious that inactivation of MALAT1 results in inhibition of osteosarcoma cell proliferation and invasion and induces the apoptotic machinery. Therefore, MALAT1 might be used as specific therapeutic target to inhibit osteosarcoma progression.

MF12 is another lncRNA that is overexpressed in osteosarcoma human tissue samples and is associated with cell proliferation, migration, and invasion in osteosarcoma cell lines MG63 and Saos-2. It promotes osteosarcoma cell growth and enhances invasiveness through regulation of FOXP4 [166]. In this context, targeting MF12 could reduce osteosarcoma growth and clinical progression.

Neuroblastoma-associated transcript 1 (NBAT1) has been found to be downregulated in osteosarcoma human samples and various osteosarcoma cell lines such as MG-63, KHOA, U2OS, LM7, and 143b [248]. Clinically, low expression levels of NBAT1 are associated with osteosarcoma metastatic dissemination and unfavorable prognosis. NBAT1 knockdown or silencing leads to enhanced osteosarcoma tumor growth, cell proliferation, migration and invasion in vitro. Induction of NBAT1, in order to be overexpressed in vitro, results in the opposite effects. It has also been demonstrated that NBAT1 positively regulates the transcription of PTEN, PDCD4 and RECK, which act as tumor suppressor, and cell death and metastasis suppressor genes, respectively, through miR-21 inactivation. Overexpression of miR-21 leads to the opposite effect [248]. Thus, NBAT1 mimics might be used to reduce osteosarcoma growth and metastatic ability.

p21-associated ncRNA DNA damage activated (PANDA) is a lncRNA which is overexpressed in osteosarcoma tissue specimens and osteosarcoma cell lines [249]. Its expression is induced up to 40-fold by DNA damage related to doxorubicin and etoposide treatment and is positively regulated by p53. PANDA is involved in positive regulation of the osteosarcoma cell cycle through p18 associated transcriptional repression. Moreover, PANDA silencing results in cell cycle arrest in G1/S transition through upregulation of cyclin-dependent kinase inhibitor p18 in U2OS osteosarcoma cell line. Depletion of PANDA leads to cell death of doxorubicin treated cells through upregulation of apoptotic activators APAF1, BIK, FAS, and LRDD [249, 250]. Taken together, these findings imply that inhibition of PANDA might serve as a therapeutic intervention to induce cell cycle arrest and apoptosis in osteosarcoma.

PVT1 is another lncRNA that is overexpressed in osteosarcoma cell lines and tissue specimens, and its upregulation is correlated with decreased survival in osteosarcoma patients. PVT1 overexpression is associated with osteosarcoma cell proliferation, migration, and invasion, and silencing of its function via siRNA has the opposite effects and promotes apoptosis and cell cycle arrest as well. Moreover, silencing of PVT1 by siRNA leads to downregulation of BCL2, CCND1, and FASN expressions through miR-195 in osteosarcoma cells [251]. PVT1 is also involved in the Warburg effect in osteosarcoma cells by promoting anaerobic glycolysis and tumor progression through regulation of the miR-497/HK2 axis [252]. Taken together, PVT1 could serve as a target in the therapeutic management of osteosarcoma.

TP73 antisense RNA 1 (TP73-AS1) is a novel oncogenic long noncoding RNA which is significantly overexpressed in osteosarcoma tissue samples and cell lines. Moreover, high expression of TP73-AS1 is correlated with advanced clinical stage, large tumor size, high metastatic potential, and poor overall survival [253]. TP73-AS1 overexpression promotes osteosarcoma cell proliferation, migration, and invasion by acting as a sponge for miR-142 to positively regulate Rac1 function [254]. TP73-AS1 might constitute a potential therapeutic target in the treatment of osteosarcoma.

All the above mentioned lncRNAs are summarized in **Table 2**.

Different methods and approaches could be used to inhibit or mimic the function of lncRNAs for therapeutic purposes, such as small molecule inhibitors, inhibiting micropeptides; RNA interference silencing by small interfering RNAs (siRNAs); or short hairpin RNAs (shRNAs), antisense oligonucleotide targeting; ribozyme, deoxyribozyme, plasmid, or viral vector-based targeting; and gene editing by CRISPR/Cas9 system [255].

In addition, a variety of delivery vehicles or carriers have been developed in an effort to target lncRNAs, such as peptide nucleic acid (PNA), lipid-based nanocarriers, poly(lactic-co-glycolic acid nanoparticles (PLGA), poly(amine-co-ester) tetrapolymers (PACE), and pHlow insertion peptides (pHLIP) [256].

Several preclinical and phase I/II clinical trials have been initiated by using the abovementioned approaches, such as the use of plasmid BC-819 expressing diphtheria toxin under the control of H19 lncRNA promoter to induce tumor reduction after intratumoral injection in order to treat bladder, ovarian, and pancreatic carcinomas [256]. Modified oligonucleotides which target antisense lncRNAs, also referred as AntagoNATs, have been tested *in vitro* and *in vivo* to modulate lncRNA expression. Administration of antisense oligonucleotides (ASOs) against MALAT1 effectively achieved inhibition of lung cancer tumor growth in mice xenografts [257]. Although ASO therapeutic approaches are promising, major obstacles, such as inadequate intracellular uptake or chemical toxicity, should be considered and taken into account. It should also be noted that although lncRNAs are regulated by *cis* or *trans* mechanisms targeting specific genes, putative effects on global gene expression should be very carefully considered.

9. Conclusions and future perspectives

In this chapter, we reviewed the involvement of lncRNAs in the pathogenesis, metastatic process, and drug resistance of osteosarcoma and summarized in **Tables 1** and **2**. We also summarized the possible roles of lncRNAs as prognostic and predictive biomarkers and their putative usefulness as therapeutic targets in osteosarcoma clinical management. However, more studies are needed to further elucidate and confirm the precise molecular mechanisms underlying these effects along with translational research in osteosarcoma metastasis and drug resistance. Translational studies are crucial in understanding if lncRNA modulation is applicable in the clinical setting and beneficial for the patients. Considering the difficulty to get osteosarcoma tissue samples at different stages of disease, it would be useful to detect lncRNA expression levels in body fluids, such as plasma or urine, providing a real-time monitoring of osteosarcoma progression [45, 258].

Studies of structural biology are also needed in order to determine the secondary and tertiary structures of lncRNAs and elucidate the molecular interactions with other biomolecules. Structural studies could provide useful knowledge for designing lncRNA mimics or pharmaceutical agents against them.

Future research should also focus on better understanding the cross-talk between different signaling pathways related to osteosarcoma development and the role of lncRNAs in these molecular interactions.

We anticipate that lncRNA-based diagnostic approaches and therapeutic interventions will be more efficient in treating this debilitating tumor and will offer significant benefit for osteosarcoma patients.

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References

- [1] Messerschmitt PJ, Garcia RM, Abdul-Karim FW, Greenfield EM, Getty PJ. Osteosarcoma. *The Journal of the American Academy of Orthopaedic Surgeons*. 2009;**17**:515-527
- [2] Ottaviani G, Jaffe N. The epidemiology of osteosarcoma. *Cancer Treatment and Research*. 2009;**152**:3-13. DOI: 10.1007/978-1-4419-0284-9_1
- [3] Mirabello L, Troisi RJ, Savage SA. International osteosarcoma incidence patterns in children and adolescents, middle ages and elderly persons. *International Journal of Cancer*. 2009; **125**:229-234. DOI: 10.1002/ijc.24320
- [4] Trihia H, Valavanis C. Histopathology and molecular pathology of bone and extraskelatal osteosarcomas. In: Manish A, editor. *Osteosarcoma*. Rijeka, Croatia: IntechOpen; 2012. pp. 3-40. DOI: 10.5772/31431
- [5] Bousquet M, Noirot C, Accadbled F, Sales de Gauzy J, Castex MP, Brousset P, et al. Whole-exome sequencing in osteosarcoma reveals important heterogeneity of genetic alterations. *Annals of Oncology*. 2016;**27**:738-744. DOI: 10.1093/annonc/mdw009
- [6] Smida J, Baumhoer D, Rosemann M, et al. Genomic alterations and allelic imbalances are strong prognostic predictors in osteosarcoma. *Clinical Cancer Research*. 2010;**16**(16): 4256-4267. DOI: 10.1158/1078-0432.CCR-10-0284
- [7] Stock C, Kager L, Fink FM, Gardner H, Ambros PF. Chromosomal regions involved in the pathogenesis of osteosarcomas. *Genes, Chromosomes & Cancer*. 2000;**28**:329-336
- [8] Atiye J, Wolf M, Kaur S, Monni O, Bohling T, Kivioja A, et al. Gene amplifications in osteosarcoma-CGH microarray analysis. *Genes, Chromosomes & Cancer*. 2005;**42**: 158-163. DOI: 10.1002/gcc.20120
- [9] Liu JJ, Liu S, Wang JG, Zhu W, Hua YQ, Sun W, et al. Telangiectatic osteosarcoma: A review of literature. *OncoTargets and Therapy*. 2013;**6**: 593-602. DOI: 10.2147/OTT.S41351
- [10] Fletcher CDM, Hogendoorn Pancras CW, Mertens F, Bridge J. *WHO Classification of Tumours of Soft Tissue and Bone (Medicine)*. 4th ed. WHO; Lyon, France: IARC Press; 2013. pp. 264-285. ISBN-10: 9283224345
- [11] Sathiyamoorthy S, Ali SZ. Osteoblastic osteosarcoma: Cytomorphologic characteristics and differential diagnosis on fine-needle aspiration. *Acta Cytologica*. 2012;**56**: 481-486
- [12] Miller BJ, Cram P, Lynch CF, Buckwalter JA. Risk factors for metastatic disease at presentation with osteosarcoma: An analysis of the SEER database. *The Journal of Bone and Joint Surgery. American Volume*. 2013;**95** (e89):1-8. DOI: 10.2106/JBJS.L.01189
- [13] Bielack S, Carrle D, Casali PG, Group EGW. Osteosarcoma: ESMO clinical recommendations for diagnosis, treatment and follow-up. *Annals of Oncology*. 2009;**20**:137-139. DOI: 10.1093/annonc/mdp154
- [14] Eilber F, Giuliano A, Eckardt J, Patterson K, Moseley S, Goodnight J. Adjuvant chemotherapy for osteosarcoma: A randomized prospective trial. *Journal of Clinical Oncology*. 1987;**5**:21-26
- [15] Allison DC, Carney SC, Ahlmann ER, Hendifar A, Chawla S, Fedenko A, et al. A meta-analysis of osteosarcoma outcomes in the modern medical era. *Sarcoma*. 2012;**2012**:704872

- [16] Meyers PA, Heller G, Healey JH, Huvos A, Applewhite A, Sun M, et al. Osteogenic sarcoma with clinically detectable metastasis at initial presentation. *Journal of Clinical Oncology*. 1993;**11**:449-453
- [17] Kager L, Zoubek A, Pötschger U, Kastner U, Flege S, Kempf-Bielack B, et al. Cooperative German-Austrian-Swiss Osteosarcoma Study Group. Primary metastatic osteosarcoma: Presentation and outcome of patients treated on neoadjuvant Cooperative Osteosarcoma Study Group protocols. *Journal of Clinical Oncology*. 2003;**21**:2011-2018. DOI: 10.1200/JCO.2003.08.132
- [18] Daw NC, Billups CA, Rodriguez-Galindo C, McCarville MB, Rao BN, Cain AM, et al. Metastatic osteosarcoma. *Cancer*. 2006;**106**:403-412
- [19] Diamantopoulos MA, Tsiakanikas P, Scorilas A. Non-coding RNAs: The riddle of the transcriptome and their perspectives in cancer. *Annals of Translational Medicine*. 2018;**6**(12):241. DOI: 10.21037/atm.2018.06.10
- [20] Kunej T, Obsteter J, Pogacar Z, Horvat S, Calin GA. The decalog of long non-coding RNA involvement in cancer diagnosis and monitoring. *Critical Reviews in Clinical Laboratory Sciences*. 2014;**51**(6):344-357. DOI: 10.3109/10408363.2014.944299
- [21] Crea F, Clermont PL, Parolia A, Wang Y, Helgason CD. The non-coding transcriptome as a dynamic regulator of cancer metastasis. *Cancer Metastasis Reviews*. 2013;**33**(1):1-16. DOI: 10.1007/s10555-013-9455-3
- [22] Cech TR, Steitz JA. The noncoding RNA revolution—Trashing old rules to forge new ones. *Cell*. 2014;**157**(1):77-94. DOI: 10.1016/j.cell.2014.03.008
- [23] Sandberg K, Samson WK, Ji H. Decoding noncoding RNA: The long and short of it. *Circulation Research*. 2013; **113**(3):240-241. DOI: 10.1161/CIRCRESAHA.113.301865
- [24] Kawaguchi T, Tanigawa A, Naganuma T, et al. SWI/SNF chromatin-remodeling complexes function in noncoding RNA-dependent assembly of nuclear bodies. *Proceedings of the National Academy of Sciences of the United States of America*. 2015; **112**(14):4304-4309. DOI: 10.1073/pnas.1423819112
- [25] Zhu Y, Rowley MJ, Böhmendorfer G, Wierzbicki AT. A SWI/SNF chromatin-remodeling complex acts in noncoding RNA-mediated transcriptional silencing. *Molecular Cell*. 2013;**49**(2):298-309. DOI: 10.1016/j.molcel.2012.11.011
- [26] Tang Y, Wang J, Lian Y, et al. Linking long non-coding RNAs and SWI/SNF complexes to chromatin remodeling in cancer. *Molecular Cancer*. 2017;**16**(1):42. DOI: 10.1186/s12943-017-0612-0
- [27] St Laurent G, Wahlestedt C, Kapranov P. The landscape of long noncoding RNA classification. *Trends in Genetics*. 2015;**31**(5):239-251. DOI: 10.1016/j.tig.2015.03.007
- [28] Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. *Cell*. 2009;**136**:629-641. DOI: 10.1016/j.cell.2009.02.006
- [29] Batista PJ, Chang HY. Long noncoding RNAs: Cellular address codes in development and disease. *Cell*. 2013; **152**(6):1298-1307. DOI: 10.1016/j.cell.2013.02.012
- [30] Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. *Molecular Cell*. 2011;**43**:904-914. DOI: 10.1016/j.molcel.2011.08.018
- [31] Ma L, Bajic VB, Zhang Z. On the classification of long non-coding RNAs.

- RNA Biology. 2013;**10**(6):925-933. DOI: 10.4161/rna.24604
- [32] Gibb EA, Brown CJ, Lam WL. The functional role of long non-coding RNA in human carcinomas. *Molecular Cancer*. 2011;**10**:38. DOI: 10.1186/1476-4598-10-38
- [33] El Khodiry A, Afify M, El Tayebi HM. Behind the curtain of non-coding RNAs; long non-coding RNAs regulating hepatocarcinogenesis. *World Journal of Gastroenterology*. 2018; **24**(5):549-572. DOI: 10.3748/wjg.v24.i5.549
- [34] Ragusa M, Barbagallo C, Statello L, et al. Non-coding landscapes of colorectal cancer. *World Journal of Gastroenterology*. 2015;**21**(41): 11709-11739. DOI: 10.3748/wjg.v21.i41.11709
- [35] Heery R, Finn SP, Cuffe S, Gray SG. Long non-coding RNAs: Key regulators of epithelial-mesenchymal transition, tumour drug resistance and cancer stem cells. *Cancers (Basel)*. 2017;**9**(4):38. DOI: 10.3390/cancers9040038
- [36] Cerk S, Schwarzenbacher D, Adiprasito JB, et al. Current status of long non-coding RNAs in human breast cancer. *International Journal of Molecular Sciences*. 2016;**17**(9):1485. DOI: 10.3390/ijms17091485
- [37] Gulia C, Baldassarra S, Signore F, et al. Role of non-coding RNAs in the etiology of bladder cancer. *Genes (Basel)*. 2017;**8**(11):339. DOI: 10.3390/genes8110339
- [38] Silva A, Bullock M, Calin G. The clinical relevance of long non-coding RNAs in cancer. *Cancers (Basel)*. 2015; **7**(4):2169-2182. DOI: 10.3390/cancers7040884
- [39] Huarte M. The emerging role of lncRNAs in cancer. *Nature Medicine*. 2015;**21**(11):1253-1261. DOI: 10.1038/nm.3981
- [40] Schmitt AM, Chang HY. Long noncoding RNAs in cancer pathways. *Cancer Cell*. 2016;**29**(4):452-463. DOI: 10.1016/j.ccell.2016.03.010
- [41] Corrà F, Agnoletto C, Minotti L, Baldassari F, Volinia S. The network of non-coding RNAs in cancer drug resistance. *Frontiers in Oncology*. 2018; **8**:327. DOI: 10.3389/fonc.2018.00327
- [42] Chen R, Wang G, Zheng Y, Hua Y, Cai Z. Long non-coding RNAs in osteosarcoma. *Oncotarget*. 2017;**8**(12): 20462-20475. DOI: 10.18632/oncotarget.14726
- [43] Min L, Garbutt C, Tu C, Hornicek F, Duan Z. Potentials of long noncoding RNAs (LncRNAs) in sarcoma: From biomarkers to therapeutic targets. *International Journal of Molecular Sciences*. 2017;**18**(4):731. DOI: 10.3390/ijms18040731
- [44] Yang Z, Li X, Yang Y, He Z, Qu X, Zhang Y. Long noncoding RNAs in the progression, metastasis, and prognosis of osteosarcoma. *Cell Death & Disease*. 2016;**7**(9):e2389. DOI: 10.1038/cddis.2016.272
- [45] Smolle MA, Pichler M. The role of long non-coding RNAs in osteosarcoma. *Noncoding RNA*. 2018;**4**(1):7. DOI: 10.3390/ncrna4010007
- [46] Lin YH, Jewell BE, Gingold J, et al. Osteosarcoma: Molecular pathogenesis and iPSC modeling. *Trends in Molecular Medicine*. 2017;**23**(8):737-755. DOI: 10.1016/j.molmed.2017.06.004
- [47] Morrow JJ, Khanna C. Osteosarcoma genetics and epigenetics: Emerging biology and candidate therapies. *Critical Reviews in Oncogenesis*. 2015;**20**(3-4):173-197. DOI: 10.1615/CritRevOncog.2015013713

- [48] Peng WX, Koirala P, Mo YY. LncRNA-mediated regulation of cell signaling in cancer. *Oncogene*. 2017; **36**(41):5661-5667. DOI: 10.1038/onc.2017.184
- [49] Yao Z, Han L, Chen Y, et al. Hedgehog signalling in the tumourigenesis and metastasis of osteosarcoma, and its potential value in the clinical therapy of osteosarcoma. *Cell Death & Disease*. 2018; **9**(6):701. DOI: 10.1038/s41419-018-0647-1
- [50] Mao X, Su Z, Mookhtiar AK. Long non-coding RNA: A versatile regulator of the nuclear factor- κ B signalling circuit. *Immunology*. 2017; **150**(4): 379-388. DOI: 10.1111/imm.12698
- [51] Wang Y, Li YP, Paulson C, et al. Wnt and the Wnt signaling pathway in bone development and disease. *Frontiers in Bioscience (Landmark Ed)*. 2014; **19**:379-407. <http://dx.doi.org/10.2741/4214>
- [52] Boland GM, Perkins G, Hall DJ, Tuan RS. Wnt 3a promotes proliferation and suppresses osteogenic differentiation of adult human mesenchymal stem cells. *Journal of Cellular Biochemistry*. 2004; **93**(6): 1210-1230. DOI: 10.1002/jcb.20284
- [53] Haydon RC, Luu HH, He TC. Osteosarcoma and osteoblastic differentiation: A new perspective on oncogenesis. *Clinical Orthopaedics and Related Research*. 2007; **454**: 237-246. DOI: 10.1097/BLO.0b013e31802b683c
- [54] Mortus JR, Zhang Y, Hughes DP. Developmental pathways hijacked by osteosarcoma. *Advances in Experimental Medicine and Biology*. 2014; **804**:93-118. DOI: 10.1007/978-3-319-04843-7_5
- [55] Lin CH, Ji T, Chen CF, Hoang BH. Wnt signaling in osteosarcoma. *Advances in Experimental Medicine and Biology*. 2014; **804**:33-45. DOI: 10.1007/978-3-319-04843-7_2
- [56] Li C, Shi X, Zhou G, Liu X, Wu S, Zhao J. The canonical Wnt-beta-catenin pathway in development and chemotherapy of osteosarcoma. *Frontiers in Bioscience (Landmark Ed)*. 2013; **18**:1384-1391. <http://dx.doi.org/10.2741/4187>
- [57] Du X, Yang J, Yang D, Tian W, Zhu Z. The genetic basis for inactivation of Wnt pathway in human osteosarcoma. *BMC Cancer*. 2014; **14**:450. DOI: 10.1186/1471-2407-14-450
- [58] Patanè S, Avnet S, Coltella N, Costa B, Sponza S, Olivero M, et al. MET overexpression turns human primary osteoblasts into osteosarcomas. *Cancer Research*. 2006; **66**(9):4750-4757. DOI: 10.1158/0008-5472.CAN-05-4422
- [59] Fazi B, Garbo S, Toschi N, et al. The lncRNA H19 positively affects the tumorigenic properties of glioblastoma cells and contributes to NKD1 repression through the recruitment of EZH2 on its promoter. *Oncotarget*. 2018; **9**(21):15512-15525. DOI: 10.18632/oncotarget.24496
- [60] Li T, Zhu J, Wang X, Chen G, Sun L, Zuo S, et al. Long non-coding RNA lncTCF7 activates the Wnt/ β -catenin pathway to promote metastasis and invasion in colorectal cancer. *Oncology Letters*. 2017; **14**(6):7384-7390. DOI: 10.3892/ol.2017.7154
- [61] Wang Y, He L, Du Y, Zhu P, Huang G, Luo J, et al. The long noncoding RNA lncTCF7 promotes self-renewal of human liver cancer stem cells through activation of Wnt signaling. *Cell Stem Cell*. 2015; **16**(4):413-425. DOI: 10.1016/j.stem.2015.03.003
- [62] Gorojankina T. Hedgehog signaling pathway: A novel model and molecular

- mechanisms of signal transduction. *Cellular and Molecular Life Sciences*. 2016;**73**(7):1317-1332. DOI: 10.1007/s00018-015-2127-4
- [63] Choudhry Z, Rikani AA, Choudhry AM, et al. Sonic hedgehog signalling pathway: A complex network. *Annals of Neurosciences*. 2014;**21**(1):28-31. DOI: 10.5214/ans.0972.7531.210109
- [64] Armas-López L, Zúñiga J, Arrieta O, Ávila-Moreno F. The Hedgehog-Gli pathway in embryonic development and cancer: Implications for pulmonary oncology therapy. *Oncotarget*. 2017;**8**(36):60684-60703. DOI: 10.18632/oncotarget.19527
- [65] Briscoe J, Théron PP. The mechanisms of Hedgehog signalling and its roles in development and disease. *Nature Reviews. Molecular Cell Biology*. 2013;**14**(7):416-429. DOI: 10.1038/nrm3598
- [66] Xavier GM, Seppala M, Barrell W, Birjandi AA, Geoghegan F, Cobourne MT. Hedgehog receptor function during craniofacial development. *Developmental Biology*. 2016;**415**(2):198-215. DOI: 10.1016/j.ydbio.2016.02.009
- [67] Lo WW, Pinnaduwa D, Gokgoz N, Wunder JS, Andrulis IL. Aberrant hedgehog signaling and clinical outcome in osteosarcoma. *Sarcoma*. 2014;**2014**:261804. DOI: 10.1155/2014/261804
- [68] Lo WW, Wunder JS, Dickson BC, Campbell V, McGovern K, Alman BA, et al. Involvement and targeted intervention of dysregulated Hedgehog signaling in osteosarcoma. *Cancer*. 2014;**120**(4):537-547. DOI: 10.1002/cncr.28439
- [69] Chan LH, Wang W, Yeung W, Deng Y, Yuan P, Mak KK. Hedgehog signaling induces osteosarcoma development through Yap1 and H19 overexpression. *Oncogene*. 2014;**33**(40):4857-4866. DOI: 10.1038/onc.2013.433
- [70] Verheyen EM. Opposing effects of Wnt and MAPK on BMP/Smad signal duration. *Developmental Cell*. 2007;**13**(6):755-756. DOI: 10.1016/j.devcel.2007.11.006
- [71] Wang CL, Xiao F, Wang CD, Zhu JF, Shen C, Zuo B, et al. Gremlin2 suppression increases the BMP-2-induced osteogenesis of human bone marrow-derived mesenchymal stem cells via the BMP-2/Smad/Runx2 signaling pathway. *Journal of Cellular Biochemistry*. 2017;**118**(2):286-297. DOI: 10.1002/jcb.25635
- [72] Rahman MS, Akhtar N, Jamil HM, Banik RS, Asaduzzaman SM. TGF- β /BMP signaling and other molecular events: Regulation of osteoblastogenesis and bone formation. *Bone Research*. 2015;**3**:15005. DOI: 10.1038/boneres.2015.5
- [73] Chen G, Deng C, Li YP. TGF- β and BMP signaling in osteoblast differentiation and bone formation. *International Journal of Biological Sciences*. 2012;**8**(2):272-288. DOI: 10.7150/ijbs.2929
- [74] Zuo C, Wang Z, Lu H, Dai Z, Liu X, Cui L. Expression profiling of lncRNAs in C3H10T1/2 mesenchymal stem cells undergoing early osteoblast differentiation. *Molecular Medicine Reports*. 2013 Aug;**8**(2):463-467. DOI: 10.3892/mmr.2013.1540
- [75] Zhu L, Xu PC. Downregulated lncRNA-ANCR promotes osteoblast differentiation by targeting EZH2 and regulating Runx2 expression. *Biochemical and Biophysical Research Communications*. 2013;**432**(4):612-617. DOI: 10.1016/j.bbrc.2013.02.036
- [76] Nguyen A, Scott MA, Dry SM, James AW. Roles of bone morphogenetic protein signaling in

osteosarcoma. *International Orthopaedics*. 2014;**38**(11):2313-2322. DOI: 10.1007/s00264-014-2512-x

[77] Wang S, Ren T, Jiao G, et al. BMPR2 promotes invasion and metastasis via the RhoA-ROCK-LIMK2 pathway in human osteosarcoma cells. *Oncotarget*. 2017;**8**(35):58625-58641. DOI: 10.18632/oncotarget.17382

[78] Guan G, Zhang Y, Lu Y, Liu L, Shi D, Wen Y, et al. The HIF-1 α /CXCR4 pathway supports hypoxia-induced metastasis of human osteosarcoma cells. *Cancer Letters*. 2015;**357**(1):254-264. DOI: 10.1016/j.canlet.2014.11.034

[79] Zhao H, Wu Y, Chen Y, Liu H. Clinical significance of hypoxia-inducible factor 1 and VEGF-A in osteosarcoma. *International Journal of Clinical Oncology*. 2015;**20**(6):1233-1243. DOI: 10.1007/s10147-015-0848-x

[80] Wang Y, Yao J, Meng H, et al. A novel long non-coding RNA, hypoxia-inducible factor-2 α promoter upstream transcript, functions as an inhibitor of osteosarcoma stem cells in vitro. *Molecular Medicine Reports*. 2014;**11**(4):2534-2540. DOI: 10.3892/mmr.2014.3024

[81] Xu Y, Wang S, Tang C, Chen W. Upregulation of long non-coding RNA HIF 1 α -anti-sense 1 induced by transforming growth factor- β -mediated targeting of sirtuin 1 promotes osteoblastic differentiation of human bone marrow stromal cells. *Molecular Medicine Reports*. 2015;**12**(5):7233-7238. DOI: 10.3892/mmr.2015.4415

[82] Tao J, Jiang MM, Jiang L, et al. Notch activation as a driver of osteogenic sarcoma. *Cancer Cell*. 2014;**26**(3):390-401. DOI: 10.1016/j.ccr.2014.07.023

[83] Li YS, Deng ZH, Zeng C, Lei GH. JNK pathway in osteosarcoma: Pathogenesis and therapeutics. *Journal*

of Receptor and Signal Transduction Research. 2016;**36**(5):465-470. DOI: 10.3109/10799893.2015.1122045

[84] Zhang J, Yu XH, Yan YG, Wang C, Wang WJ. PI3K/Akt signaling in osteosarcoma. *Clinica Chimica Acta*. 2015;**444**:182-192. DOI: 10.1016/j.cca.2014.12.041

[85] Adamopoulos C, Gargalionis AN, Basdra EK, Papavassiliou AG. Deciphering signaling networks in osteosarcoma pathobiology. *Experimental Biology and Medicine (Maywood, N.J.)*. 2016;**241**(12):1296-1305. DOI: 10.1177/1535370216648806

[86] Li JP, Liu LH, Li J, Chen Y, Jiang XW, Ouyang YR, et al. Microarray expression profile of long noncoding RNAs in human osteosarcoma. *Biochemical and Biophysical Research Communications*. 2013;**433**(2):200-206. DOI: 10.1016/j.bbrc.2013.02.083

[87] Dong Y, Liang G, Yuan B, Yang C, Gao R, Zhou X. MALAT1 promotes the proliferation and metastasis of osteosarcoma cells by activating the PI3K/Akt pathway. *Tumour Biology*. 2015;**36**(3):1477-1486. DOI: 10.1007/s13277-014-2631-4

[88] Cai X, Liu Y, Yang W, Xia Y, Yang C, Yang S, et al. Long noncoding RNA MALAT1 as a potential therapeutic target in osteosarcoma. *Journal of Orthopaedic Research*. 2016;**34**(6):932-941. DOI: 10.1002/jor.23105

[89] Qian M, Yang X, Li Z, Jiang C, Song D, Yan W, et al. P50-associated COX-2 extragenic RNA (PACER) overexpression promotes proliferation and metastasis of osteosarcoma cells by activating COX-2 gene. *Tumour Biology*. 2016;**37**(3):3879-3886. DOI: 10.1007/s13277-015-3838-8

[90] Feng ZM, Guo SM. Tim-3 facilitates osteosarcoma proliferation and metastasis through the NF- κ B pathway and epithelial-mesenchymal transition.

Genetics and Molecular Research. 2016;**15**(3):7844. DOI: 10.4238/gmr.15037844

[91] Gong T, Su X, Xia Q, Wang J, Kan S. Expression of NF- κ B and PTEN in osteosarcoma and its clinical significance. *Oncology Letters*. 2017; **14**(6):6744-6748. DOI: 10.3892/ol.2017.6972

[92] Arshad A, Lifang H, Airong Q, Chu C, Tuanmin Y. Long noncoding RNAs and human osteosarcoma. *Journal of Stem Cell Research and Therapy*. 2018; **8**(3):418. DOI: 10.4172/2157-7633.1000418

[93] Gabory A, Jammes H, Dandolo L. The H19 locus: Role of an imprinted non-coding RNA in growth and development. *BioEssays*. 2010;**32**: 473-480. DOI: 10.1002/bies.200900170

[94] Berteaux N, Aptel N, Cathala G, et al. A novel H19 antisense RNA overexpressed in breast cancer contributes to paternal IGF2 expression. *Molecular and Cellular Biology*. 2008; **28**(22):6731-6745. DOI: 10.1128/MCB.02103-07

[95] Tran VG, Court F, Duputié A, et al. H19 antisense RNA can up-regulate IGF2 transcription by activation of a novel promoter in mouse myoblasts. *PLoS One*. 2012;**7**(5):e37923. DOI: 10.1371/journal.pone.0037923

[96] Dunn KL, Davie JR. The many roles of the transcriptional regulator CTCF. *Biochemistry and Cell Biology*. 2003;**81**(3):161-167. DOI: 10.1139/o03-052

[97] Ulaner GA, Vu TH, Li T, Hu JF, Yao XM, Yang Y, et al. Loss of imprinting of IGF2 and H19 in osteosarcoma is accompanied by reciprocal methylation changes of a CTCF-binding site. *Human Molecular Genetics*. 2003;**12**(5):535-549. DOI: 10.1093/hmg/ddg034

[98] Xia WK, Lin QF, Shen D, Liu ZL, Su J, Mao WD. Clinical implication of long noncoding RNA 91H expression profile in osteosarcoma patients. *OncoTargets and Therapy*. 2016;**9**:4645-4652. DOI: 10.2147/OTT.S103376

[99] Zhu L, Xu PC. Downregulated LncRNA-ANCR promotes osteoblast differentiation by targeting EZH2 and regulating Runx2 expression. *Biochemical Biophysical Research Communications*. 2013;**432**(4):612-617. DOI: 10.1016/j.bbrc.2013.02.036

[100] Zhang F, Peng H. LncRNA-ANCR regulates the cell growth of osteosarcoma by interacting with EZH2 and affecting the expression of p21 and p27. *Journal of Orthopaedic Surgery and Research*. 2017;**12**(1):103. DOI: 10.1186/s13018-017-0599-7

[101] Min L, Hong S, Duan H, Zhou Y, Zhang W, Luo Y, et al. Antidifferentiation noncoding RNA regulates the proliferation of osteosarcoma cells. *Cancer Biotherapy & Radiopharmaceuticals*. 2016;**31**(2):52-57. DOI: 10.1089/cbr.2015.1888

[102] Godinho M, Meijer D, Setyono-Han B, Dorssers LC, van Agthoven T. Characterization of BCAR4, a novel oncogene causing endocrine resistance in human breast cancer cells. *Journal of Cellular Physiology*. 2011;**226**(7): 1741-1749. DOI: 10.1002/jcp.22503

[103] Godinho MF, Sieuwerts AM, Look MP, et al. Relevance of BCAR4 in tamoxifen resistance and tumour aggressiveness of human breast cancer. *British Journal of Cancer*. 2010;**103**(8): 1284-1291. DOI: 10.1038/sj.bjc.6605884

[104] Chen F, Mo J, Zhang L. Long noncoding RNA BCAR4 promotes osteosarcoma progression through activating GLI2-dependent gene transcription. *Tumour Biology*. 2016; **37**(10):13403-13412. DOI: 10.1007/s13277-016-5256-y

- [105] Ju L, Zhou YM, Yang GS. Up-regulation of long non-coding RNA BCAR4 predicts a poor prognosis in patients with osteosarcoma, and promotes cell invasion and metastasis. *European Review for Medical and Pharmacological Sciences*. 2016;**20**(21): 4445-4451
- [106] Li Z, Dou P, Liu T, He S. Application of long noncoding RNAs in osteosarcoma: biomarkers and therapeutic targets. *Cellular Physiology and Biochemistry*. 2017;**42**(4): 1407-1419. DOI: 10.1159/000479205
- [107] Sun J, Wang X, Fu C, Zou J, Hua H, Bi Z. Long noncoding RNA FGFR3-AS1 promotes osteosarcoma growth through regulating its natural antisense transcript FGFR3. *Molecular Biology Reports*. 2016;**43**:427-436. DOI: 10.1007/s11033-016-3975-1
- [108] Li W, He X, Xue R, Zhang Y, Zhang X, Lu J, et al. Combined over-expression of the hypoxia-inducible factor 2 α gene and its long non-coding RNA predicts unfavorable prognosis of patients with osteosarcoma. *Pathology, Research and Practice*. 2016;**212**: 861-866. DOI: 10.1016/j.prp.2016.06.013
- [109] Preker P, Almvig K, Christensen MS, Valen E, Mapendano CK, Sandelin A, et al. PROMoter uPstream transcripts share characteristics with mRNAs and are produced upstream of all three major types of mammalian promoters. *Nucleic Acids Research*. 2011;**39**:7179-7193. DOI: 10.1093/nar/gkr370
- [110] Taft RJ, Kaplan CD, Simons C, Mattick JS. Evolution, biogenesis and function of promoter-associated RNAs. *Cell Cycle*. 2009;**8**:2332-2338. DOI: 10.4161/cc.8.15.9154
- [111] Albrecht AS, Ørom UA. Bidirectional expression of long ncRNA/protein-coding gene pairs in cancer. *Briefings in Functional Genomics*. 2016;**15**(3):167-173. DOI: 10.1093/bfpg/elv048
- [112] Yao J, Li J, Geng P, Li Y, Chen H, Zhu Y. Knockdown of a HIF-2 α promoter upstream long noncoding RNA impairs colorectal cancer stem cell properties in vitro through HIF-2 α downregulation. *OncoTargets and Therapy*. 2015;**8**:3467-3474. DOI: 10.2147/OTT.S81393
- [113] Wang B, Su Y, Yang Q, Lv D, Zhang W, Tang K, et al. Overexpression of long non-coding RNA HOTAIR promotes tumor growth and metastasis in human osteosarcoma. *Molecules and Cells*. 2015;**38**(5):432-440. DOI: 10.14348/molcells.2015.2327
- [114] Qiu JJ, Lin YY, Ye LC, Ding JX, Feng WW, Jin HY, et al. Overexpression of long non-coding RNA HOTAIR predicts poor patient prognosis and promotes tumor metastasis in epithelial ovarian cancer. *Gynecologic Oncology*. 2014;**134**(1):121-128. DOI: 10.1016/j.ygyno.2014.03.556
- [115] Liu XH, Liu ZL, Sun M, Liu J, Wang ZX, De W. The long non-coding RNA HOTAIR indicates a poor prognosis and promotes metastasis in non-small cell lung cancer. *BMC Cancer*. 2013;**13**:464. DOI: 10.1186/1471-2407-13-464
- [116] Xue X, Yang YA, Zhang A, et al. LncRNA HOTAIR enhances ER signaling and confers tamoxifen resistance in breast cancer. *Oncogene*. 2015;**35**(21):2746-2755. DOI: 10.1038/onc.2015.340
- [117] Wu L, Zhang L, Zheng S. Role of the long non-coding RNA HOTAIR in hepatocellular carcinoma. *Oncology Letters*. 2017;**14**(2):1233-1239. DOI: 10.3892/ol.2017.6312
- [118] Zhang J, Zhang P, Wang L, Piao HL, Ma L. Long non-coding RNA

- HOTAIR in carcinogenesis and metastasis. *Acta Biochimica et Biophysica Sinica Shanghai*. 2013;**46**(1): 1-5. DOI: 10.1093/abbs/gmt117
- [119] Tsai MC, Manor O, Wan Y, et al. Long noncoding RNA as modular scaffold of histone modification complexes. *Science*. 2010;**329**(5992): 689-693. DOI: 10.1126/science.1192002
- [120] Zhou Q, Chen F, Fei Z, et al. Genetic variants of lncRNA HOTAIR contribute to the risk of osteosarcoma. *Oncotarget*. 2016;**7**(15):19928-19934. DOI: 10.18632/oncotarget.7957
- [121] Li F, Cao L, Hang D, Wang F, Wang Q. Long non-coding RNA HOTTIP is up-regulated and associated with poor prognosis in patients with osteosarcoma. *International Journal of Clinical and Experimental Pathology*. 2015;**8**(9):11414-11420
- [122] Cheng Y, Jutooru I, Chadalapaka G, Corton JC, Safe S. The long non-coding RNA HOTTIP enhances pancreatic cancer cell proliferation, survival and migration. *Oncotarget*. 2015;**6**(13): 10840-10852. DOI: 10.18632/oncotarget.3450
- [123] Ye H, Liu K, Qian K. Overexpression of long noncoding RNA HOTTIP promotes tumor invasion and predicts poor prognosis in gastric cancer. *OncoTargets and Therapy*. 2016;**9**:2081-2088. DOI: 10.2147/OTT.S95414
- [124] Chen X, Han H, Li Y, Zhang Q, Mo K, Chen S. Upregulation of long noncoding RNA HOTTIP promotes metastasis of esophageal squamous cell carcinoma via induction of EMT. *Oncotarget*. 2016;**7**(51):84480-84485. DOI: 10.18632/oncotarget.12995
- [125] Zhang GJ, Song W, Song Y. Overexpression of HOTTIP promotes proliferation and drug resistance of lung adenocarcinoma by regulating AKT signaling pathway. *European Review for Medical and Pharmacological Sciences*. 2017;**21**(24):5683-5690. DOI: 10.26355/eurrev_201712_14013
- [126] Wang KC, Yang YW, Liu B, et al. A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. *Nature*. 2011;**472**(7341): 120-124. DOI: 10.1038/nature09819
- [127] Lian Y, Cai Z, Gong H, Xue S, Wu D, Wang K. HOTTIP: A critical oncogenic long non-coding RNA in human cancers. *Molecular BioSystems*. 2016;**12**(11):3247-3253. DOI: 10.1039/c6mb00475j
- [128] Li Z, Yu X, Shen J. Long non-coding RNAs: Emerging players in osteosarcoma. *Tumour Biology*. 2016 Mar;**37**(3):2811-2816. DOI: 10.1007/s13277-015-4749-4
- [129] Yu X, Zheng H, Chan MT, Wu WK. HULC: An oncogenic long non-coding RNA in human cancer. *Journal of Cellular and Molecular Medicine*. 2016;**21**(2):410-417. DOI: 10.1111/jcmm.12956
- [130] Hämmerle M, Gutschner T, Uckelmann H, Ozgur S, Fiskin E, Gross M, et al. Posttranscriptional destabilization of the liver-specific long noncoding RNA HULC by the IGF2 mRNA-binding protein 1 (IGF2BP1). *Hepatology*. 2013;**58**(5):1703-1712. DOI: 10.1002/hep.26537
- [131] Cui M, Xiao Z, Wang Y, Zheng M, Song T, Cai X, et al. Long noncoding RNA HULC modulates abnormal lipid metabolism in hepatoma cells through an miR-9-mediated RXRA signaling pathway. *Cancer Research*. 2015;**75**(5): 846-857. DOI: 10.1158/0008-5472.CAN-14-1192
- [132] Li SP, Xu HX, Yu Y, et al. LncRNA HULC enhances epithelial-mesenchymal transition to promote tumorigenesis and metastasis of hepatocellular carcinoma via the

miR-200a-3p/ZEB1 signaling pathway. *Oncotarget*. 2016;7(27):42431-42446. DOI: 10.18632/oncotarget.9883

[133] Yang XJ, Huang CQ, Peng CW, Hou JX, Liu JY. Long noncoding RNA HULC promotes colorectal carcinoma progression through epigenetically repressing NKD2 expression. *Gene*. 2016;592(1):172-178. DOI: 10.1016/j.gene.2016.08.002

[134] Lu Z, Xiao Z, Liu F, et al. Long non-coding RNA HULC promotes tumor angiogenesis in liver cancer by up-regulating sphingosine kinase 1 (SPHK1). *Oncotarget*. 2015;7(1):241-254. DOI: 10.18632/oncotarget.6280

[135] Sun XH, Yang LB, Geng XL, Wang R, Zhang ZC. Increased expression of lncRNA HULC indicates a poor prognosis and promotes cell metastasis in osteosarcoma. *International Journal of Clinical and Experimental Pathology*. 2015;8(3):2994-3000

[136] Uzan VR, Av L, Boldrini É, et al. High expression of HULC is associated with poor prognosis in osteosarcoma patients. *PLoS One*. 2016;11(6):e0156774. DOI: 10.1371/journal.pone.0156774

[137] Ji P, Diederichs S, Wang W, Böing S, Metzger R, Schneider PM, et al. MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer. *Oncogene*. 2003;22(39):8031-8041. DOI: 10.1038/sj.onc.1206928

[138] Gutschner T, Hämmerle M, Eissmann M, et al. The noncoding RNA MALAT1 is a critical regulator of the metastasis phenotype of lung cancer cells. *Cancer Research*. 2012;73(3):1180-1189. DOI: 10.1158/0008-5472.CAN-12-2850

[139] Chou J, Wang B, Zheng T, Li X, Zheng L, Hu J, et al. MALAT1 induced migration and invasion of human breast

cancer cells by competitively binding miR-1 with cdc42. *Biochemical and Biophysical Research Communications*. 2016;472(1):262-269. DOI: 10.1016/j.bbrc.2016.02.102

[140] Li J, Wang J, Chen Y, et al. LncRNA MALAT1 exerts oncogenic functions in lung adenocarcinoma by targeting miR-204. *American Journal of Cancer Research*. 2016;6(5):1099-1107

[141] Luan W, Li L, Shi Y, et al. Long non-coding RNA MALAT1 acts as a competing endogenous RNA to promote malignant melanoma growth and metastasis by sponging miR-22. *Oncotarget*. 2016;7(39):63901-63912. DOI: 10.18632/oncotarget.11564

[142] Huo Y, Li Q, Wang X, et al. MALAT1 predicts poor survival in osteosarcoma patients and promotes cell metastasis through associating with EZH2. *Oncotarget*. 2017;8(29):46993-47006. DOI: 10.18632/oncotarget.16551

[143] Chen D, Wang H, Zhang M, Jiang S, Zhou C, Fang B, et al. Abnormally expressed long non-coding RNAs in prognosis of Osteosarcoma: A systematic review and meta-analysis. *Journal of Bone Oncology*. 2018;13:76-90. DOI: 10.1016/j.jbo.2018.09.005

[144] Luo W, He H, Xiao W, et al. MALAT1 promotes osteosarcoma development by targeting TGFA via MIR376A. *Oncotarget*. 2016;7(34):54733-54743. DOI: 10.18632/oncotarget.10752

[145] Taniguchi M, Fujiwara K, Nakai Y, et al. Inhibition of malignant phenotypes of human osteosarcoma cells by a gene silencer, a pyrrole-imidazole polyamide, which targets an E-box motif. *FEBS Open Bio*. 2014;4:328-334. DOI: 10.1016/j.fob.2014.03.004

[146] Li Z, Shen J, Chan MT, Wu WK. TUG1: A pivotal oncogenic long

- non-coding RNA of human cancers. *Cell Proliferation*. 2016;**49**(4):471-475. DOI: 10.1111/cpr.12269
- [147] Khalil AM, Guttman M, Huarte M, et al. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. *Proceedings of the National Academy of Sciences of the United States of America*. 2009;**106**(28):11667-11672. DOI: 10.1073/pnas.0904715106
- [148] Xie CH, Cao YM, Huang Y, Shi QW, Guo JH, Fan ZW, et al. Long non-coding RNA TUG1 contributes to tumorigenesis of human osteosarcoma by sponging miR-9-5p and regulating POU2F1 expression. *Tumour Biology*. 2016 Nov;**37**(11):15031-15041. DOI: 10.1007/s13277-016-5391-5
- [149] Cao J, Han X, Qi X, Jin X, Li X. TUG1 promotes osteosarcoma tumorigenesis by upregulating EZH2 expression via miR-144-3p. *International Journal of Oncology*. 2017; **51**(4):1115-1123. DOI: 10.3892/ijo.2017.4110
- [150] Li H, Tian G, Tian F, Shao L. Long non-coding RNA TUG1 promotes osteosarcoma cell proliferation and invasion through inhibition of microRNA-212-3p expression. *Experimental and Therapeutic Medicine*. 2018;**16**(2):779-787. DOI: 10.3892/etm.2018.6216
- [151] Zhang Q, Geng PL, Yin P, Wang XL, Jia JP, Yao J. Down-regulation of long non-coding RNA TUG1 inhibits osteosarcoma cell proliferation and promotes apoptosis. *Asian Pacific Journal of Cancer Prevention*. 2013; **14**(4):2311-2315. DOI: 10.7314/APJCP.2013.14.4.2311
- [152] Ma B, Li M, Zhang L, Huang M, Lei JB, Fu GH, et al. Upregulation of long non-coding RNA TUG1 correlates with poor prognosis and disease status in osteosarcoma. *Tumour Biology*. 2016; (4):4445-4455. DOI: 10.1007/s13277-015-4301-6
- [153] Jin B, Gong Y, Li H, Jiao L, Xin D, Gong Y, et al. C/EBP β promotes the viability of human bladder cancer cell by contributing to the transcription of bladder cancer specific lncRNA UCA1. *Biochemical and Biophysical Research Communications*. 2018; pii: S0006-291X(18)32330-1. DOI: 10.1016/j.bbrc.2018.10.152
- [154] Xue M, Chen W, Li X. Urothelial cancer associated 1: A long noncoding RNA with a crucial role in cancer. *Journal of Cancer Research and Clinical Oncology*. 2016;**142**:1407-1419. DOI: 10.1007/s00432-015-2042-y
- [155] He A, Hu R, Chen Z, et al. Role of long noncoding RNA UCA1 as a common molecular marker for lymph node metastasis and prognosis in various cancers: A meta-analysis. *Oncotarget*. 2016;**8**(1):1937-1943. DOI: 10.18632/oncotarget.12463
- [156] Liu FT, Zhu PQ, Luo HL, Zhang Y, Qiu C. Prognostic value of long non-coding RNA UCA1 in human solid tumors. *Oncotarget*. 2016;**7**(36):57991-58000. DOI: 10.18632/oncotarget.11155
- [157] Wang X, Gong Y, Jin B, et al. Long non-coding RNA urothelial carcinoma associated 1 induces cell replication by inhibiting BRG1 in 5637 cells. *Oncology Reports*. 2014;**32**(3):1281-1290. DOI: 10.3892/or.2014.3309
- [158] Wang F, Ying HQ, He BS, et al. Upregulated lncRNA-UCA1 contributes to progression of hepatocellular carcinoma through inhibition of miR-216b and activation of FGFR1/ERK signaling pathway. *Oncotarget*. 2015; **6**(10):7899-7917. DOI: 10.18632/oncotarget.3219
- [159] Sun X, Haider Ali MSS, Moran M. The role of interactions of long

non-coding RNAs and heterogeneous nuclear ribonucleoproteins in regulating cellular functions. *The Biochemical Journal*. 2017;**474**(17):2925-2935. DOI: 10.1042/BCJ20170280

[160] Li T, Xiao Y, Huang T. HIF-1 α -induced upregulation of lncRNA UCA1 promotes cell growth in osteosarcoma by inactivating the PTEN/AKT signaling pathway. *Oncology Reports*. 2018;**39**(3): 1072-1080. DOI: 10.3892/or.2018.6182

[161] Li C, Liang G, Yang S, et al. Dysregulated lncRNA-UCA1 contributes to the progression of gastric cancer through regulation of the PI3K-Akt-mTOR signaling pathway. *Oncotarget*. 2017;**8**(55):93476-93491. DOI: 10.18632/oncotarget.19281

[162] Shao Y, Li H, Du R, Meng J, Yang G. Involvement of non-coding RNAs in chemotherapy resistance of ovarian cancer. *Journal of Cancer*. 2018;**9**(11): 1966-1972. Published 2018 Ap. 30. DOI: 10.7150/jca.24550

[163] Wang H, Guan Z, He K, Qian J, Cao J, Teng L. LncRNA UCA1 in anti-cancer drug resistance. *Oncotarget*. 2017;**8**(38):64638-64650. DOI: 10.18632/oncotarget.18344

[164] Liu H, Wang G, Yang L, Qu J, Yang Z, Zhou X. Knockdown of long non-coding RNA UCA1 increases the tamoxifen sensitivity of breast cancer cells through inhibition of Wnt/ β -catenin pathway. *PLoS One*. 2016; **11**(12):e0168406. DOI: 10.1371/journal.pone.0168406

[165] Li W, Xie P, Ruan WH. Overexpression of lncRNA UCA1 promotes osteosarcoma progression and correlates with poor prognosis. *Journal of Bone Oncology*. 2016;**5**(2):80-85. DOI: 10.1016/j.jbo.2016.05.003

[166] Yin Z, Ding H, He E, Chen J, Li M. Overexpression of long non-coding RNA MFI2 promotes cell proliferation

and suppresses apoptosis in human osteosarcoma. *Oncology Reports*. 2016; **36**(4):2033-2040. DOI: 10.3892/or.2016.5013

[167] Ruan W, Wang P, Feng S, Xue Y, Li Y. Long non-coding RNA small nucleolar RNA host gene 12 (SNHG12) promotes cell proliferation and migration by upregulating angiomin gene expression in human osteosarcoma cells. *Tumour Biology*. 2016;**37**(3): 4065-4073. DOI: 10.1007/s13277-015-4256-7

[168] Zhou S, Yu L, Xiong M, Dai G. LncRNA SNHG12 promotes tumorigenesis and metastasis in osteosarcoma by upregulating Notch2 by sponging miR-195-5p. *Biochemical and Biophysical Research Communications*. 2018;**495**(2):1822-1832. DOI: 10.1016/j.bbrc.2017.12.047

[169] Liu C, Lin J. Long noncoding RNA ZEB1-AS1 acts as an oncogene in osteosarcoma by epigenetically activating ZEB1. *American Journal of Translational Research*. 2016;**8**(10): 4095-4105

[170] Liu C, Pan C, Cai Y, Wang H. Interplay between long noncoding RNA ZEB1-AS1 and miR-200s regulates osteosarcoma cell proliferation and migration. *Journal of Cellular Biochemistry*. 2017;**118**(8):2250-2260. DOI: 10.1002/jcb.25879

[171] Pasic I, Shlien A, Durbin AD, Stavropoulos DJ, Baskin B, Ray PN, et al. Recurrent focal copy-number changes and loss of heterozygosity implicate two noncoding RNAs and one tumor suppressor gene at chromosome 3q13.31 in osteosarcoma. *Cancer Research*. 2010;**70**:160-171. DOI: 10.1158/0008-5472.CAN-09-1902

[172] Xie J, Lin D, Lee DH, Akunowicz J, Hansen M, Miller C, et al. Copy number analysis identifies tumor suppressive lncRNAs in human osteosarcoma.

- International Journal of Oncology. 2017; **50**(3):863-872. DOI: 10.3892/ijo.2017.3864
- [173] Liu Q, Huang J, Zhou N, et al. LncRNA loc285194 is a p53-regulated tumor suppressor. *Nucleic Acids Research*. 2013;**41**(9):4976-4987. DOI: 10.1093/nar/gkt182
- [174] Zhang A, Xu M, Mo YY. Role of the lncRNA-p53 regulatory network in cancer. *Journal of Molecular Cell Biology*. 2014;**6**(3):181-191. DOI: 10.1093/jmcb/mju013
- [175] Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: The Rosetta Stone of a hidden RNA language? *Cell*. 2011;**146**(3):353-358. DOI: 10.1016/j.cell.2011.07.014
- [176] da Rocha ST, Edwards CA, Ito M, Ogata T, Ferguson-Smith AC. Genomic imprinting at the mammalian Dlk1-Dio3 domain. *Trends in Genetics* 2008;**24**(6): 306-316. DOI: 10.1016/j.tig.2008.03.011
- [177] He Y, Luo Y, Liang B, Ye L, Lu G, He W. Potential applications of MEG3 in cancer diagnosis and prognosis. *Oncotarget*. 2017;**8**(42):73282-73295. DOI: 10.18632/oncotarget.19931
- [178] Zhou Y, Zhang X, Klibanski A. MEG3 noncoding RNA: A tumor suppressor. *Journal of Molecular Endocrinology*. 2012;**48**(3):R45-R53. DOI: 10.1530/JME-12-0008
- [179] Zhu M, Wang X, Gu Y, Wang F, Li L, Qiu X. MEG3 overexpression inhibits the tumorigenesis of breast cancer by downregulating miR-21 through the PI3K/Akt pathway. *Archives of Biochemistry and Biophysics*. 2018;**661**: 22-30. DOI: 10.1016/j.abb.2018.10.021
- [180] Zhang CY, Yu MS, Li X, Zhang Z, Han CR, Yan B. Overexpression of long non-coding RNA MEG3 suppresses breast cancer cell proliferation, invasion, and angiogenesis through AKT pathway. *Tumour Biology*. 2017;**39**(6): 1-12. DOI: 10.1177/1010428317701311
- [181] He JH, Han ZP, Liu JM, Zhou JB, Zou MX, Lv YB, et al. Overexpression of long non-coding RNA MEG3 inhibits proliferation of hepatocellular carcinoma Huh7 Cells via negative modulation of miRNA-664. *Journal of Cellular Biochemistry*. 2017;**118**(11): 3713-3721. DOI: 10.1002/jcb.26018
- [182] Sun L, Li Y, Yang B. Downregulated long non-coding RNA MEG3 in breast cancer regulates proliferation, migration and invasion by depending on p53's transcriptional activity. *Biochemical and Biophysical Research Communications*. 2016; **478**(1):323-329. DOI: 10.1016/j.bbrc.2016.05.031
- [183] Zhang J, Yao T, Wang Y, Yu J, Liu Y, Lin Z. Long noncoding RNA MEG3 is downregulated in cervical cancer and affects cell proliferation and apoptosis by regulating miR-21. *Cancer Biology & Therapy*. 2015;**17**(1): 104-113. DOI: 10.1080/15384047.2015.1108496
- [184] Lu KH, Li W, Liu XH, et al. Long non-coding RNA MEG3 inhibits NSCLC cells proliferation and induces apoptosis by affecting p53 expression. *BMC Cancer*. 2013;**13**:461. DOI: 10.1186/1471-2407-13-461
- [185] Liu Z, Wu C, Xie N, Wang P. Long non-coding RNA MEG3 inhibits the proliferation and metastasis of oral squamous cell carcinoma by regulating the WNT/ β -catenin signaling pathway. *Oncology Letters*. 2017;**14**(4): 4053-4058. DOI: 10.3892/ol.2017.6682
- [186] Zarkou V, Galaras A, Giakountis A, Hatzis P. Crosstalk mechanisms between the WNT signaling pathway and long non-coding RNAs. *Non-coding RNA Research*. 2018;**3**(2):42-53. DOI: 10.1016/j.ncrna.2018.04.001

- [187] Shi Y, Lv C, Shi L, Tu G. MEG3 inhibits proliferation and invasion and promotes apoptosis of human osteosarcoma cells. *Oncology Letters*. 2018;**15**(2):1917-1923. DOI: 10.3892/ol.2017.7463
- [188] Tian ZZ, Guo XJ, Zhao YM, Fang Y. Decreased expression of long non-coding RNA MEG3 acts as a potential predictor biomarker in progression and poor prognosis of osteosarcoma. *International Journal of Clinical and Experimental Pathology*. 2015;**8**(11): 15138-15142
- [189] Li N, Shi K, Li W. TUSC7: A novel tumor suppressor long non-coding RNA in human cancers. *Journal of Cellular Physiology*. 2018;**233**(9):6401-6407. DOI: 10.1002/jcp.26544
- [190] Cong M, Li J, Jing R, Li Z. Long non-coding RNA tumor suppressor candidate 7 functions as a tumor suppressor and inhibits proliferation in osteosarcoma. *Tumour Biology*. 2016; **37**(7):9441-9450. DOI: 10.1007/s13277-015-4414-y
- [191] Celià-Terrassa T, Kang Y. Distinctive properties of metastasis-initiating cells. *Genes & Development*. 2016;**30**(8):892-908. DOI: 10.1101/gad.277681.116
- [192] Hurst DR, Welch DR. Metastasis suppressor genes at the interface between the environment and tumor cell growth. *International Review of Cell and Molecular Biology*. 2011;**286**: 107-180. DOI: 10.1016/B978-0-12-385859-7.00003-3
- [193] Malagobadan S, Nagoor NH. Evaluation of MicroRNAs regulating anoikis pathways and its therapeutic potential. *BioMed Research International*. 2015;**2015**:716816. DOI: 10.1155/2015/716816
- [194] Cao Z, Livas T, Kyprianou N. Anoikis and EMT: Lethal "Liaisons" during cancer progression. *Critical Reviews in Oncogenesis*. 2016;**21**(3-4): 155-168. DOI: 10.1615/CritRevOncog.2016016955
- [195] Li J, Yang Z, Li Y, et al. Cell apoptosis, autophagy and necroptosis in osteosarcoma treatment. *Oncotarget*. 2016;**7**(28):44763-44778. DOI: 10.18632/oncotarget.8206
- [196] Maugg D, Rothenaigner I, Schorpp K, et al. New small molecules targeting apoptosis and cell viability in osteosarcoma. *PLoS One*. 2015;**10**(6): e0129058. DOI: 10.1371/journal.pone.0129058
- [197] He P, Zhang Z, Huang G, et al. miR-141 modulates osteoblastic cell proliferation by regulating the target gene of lncRNA H19 and lncRNA H19-derived miR-675. *American Journal of Translational Research*. 2016;**8**(4): 1780-1788
- [198] Peng ZQ, Lu RB, Xiao DM, Xiao ZM. Increased expression of the lncRNA BANCR and its prognostic significance in human osteosarcoma. *Genetics and Molecular Research*. 2016;**15**(1):7480. DOI: 10.4238/gmr.15017480
- [199] Sun Y, Qin B. Long noncoding RNA MALAT1 regulates HDAC4-mediated proliferation and apoptosis via decoying of miR-140-5p in osteosarcoma cells. *Cancer Medicine*. 2018;**7**(9):4584-4597. DOI: 10.1002/cam4.1677
- [200] Liu Q, Zhang H, Jiang X, Qian C, Liu Z, Luo D. Factors involved in cancer metastasis: A better understanding to "seed and soil" hypothesis. *Molecular Cancer*. 2017;**16**(1):176. DOI: 10.1186/s12943-017-0742-4
- [201] Lambert AW, Pattabiraman DR, Weinberg RA. Emerging biological principles of metastasis. *Cell*. 2017; **168**(4):670-691. DOI: 10.1016/j.cell.2016.11.037

- [202] Jiang C, Li X, Zhao H, Liu H. Long non-coding RNAs: Potential new biomarkers for predicting tumor invasion and metastasis. *Molecular Cancer*. 2016;**15**(1):62. DOI: 10.1186/s12943-016-0545-z
- [203] Xie L, Yao Z, Zhang Y, et al. Deep RNA sequencing reveals the dynamic regulation of miRNA, lncRNAs, and mRNAs in osteosarcoma tumorigenesis and pulmonary metastasis. *Cell Death & Disease*. 2018;**9**(7):772. DOI: 10.1038/s41419-018-0813-5
- [204] Gong J, Zhang H, He L, Wang L, Wang J. Increased expression of long non-coding RNA BCAR4 is predictive of poor prognosis in patients with non-small cell lung cancer. *The Tohoku Journal of Experimental Medicine*. 2017;**241**(1):29-34. DOI: 10.1620/tjem.241.29
- [205] Xing Z, Lin C, Yang L. Unraveling the therapeutic potential of the LncRNA-dependent noncanonical Hedgehog pathway in cancer. *Molecular & Cellular Oncology*. 2015;**2**(4):e998900. DOI: 10.1080/23723556.2014.998900
- [206] Wang Y, Zeng X, Wang N, et al. 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. *Molecular Cancer*. 2018;**17**(1):89. DOI: 10.1186/s12943-018-0837-6
- [207] Wang B, Su Y, Yang Q, Lv D, Zhang W, Tang K, Wang H, Zhang R, Liu R. Overexpression of long non-coding RNA HOTAIR promotes tumor growth and metastasis in human osteosarcoma. *Molecules and Cells* 2015;**38**(5):432-440. DOI: 10.14348/molcells.2015.2327
- [208] Xiao H, Zhang F, Zou Y, Li J, Liu Y, Huang W. The function and mechanism of long non-coding RNA-ATB in cancers. *Frontiers in Physiology*. 2018;**9**:321. DOI: 10.3389/fphys.2018.00321
- [209] Burk U, Schubert J, Wellner U, et al. A reciprocal repression between ZEB1 and members of the miR-200 family promotes EMT and invasion in cancer cells. *EMBO Reports*. 2008;**9**(6):582-589. DOI: 10.1038/embor.2008.74
- [210] Jang SY, Kim G, Park SY, et al. Clinical significance of lncRNA-ATB expression in human hepatocellular carcinoma. *Oncotarget*. 2017;**8**(45):78588-78597. DOI: 10.18632/oncotarget.21094
- [211] Yuan JH, Yang F, Wang F, Ma JZ, Guo YJ, Tao QF, et al. A long noncoding RNA activated by TGF-beta promotes the invasion-metastasis cascade in hepatocellular carcinoma. *Cancer Cell*. 2014;**25**:666-681. DOI: 10.1016/j.ccr.2014.03.010
- [212] Han F, Wang C, Wang Y, Zhang L. Long noncoding RNA ATB promotes osteosarcoma cell proliferation, migration and invasion by suppressing miR-200s. *American Journal of Cancer Research*. 2017;**7**(4):770-783
- [213] Chaturvedi MM, Sung B, Yadav VR, Kannappan R, Aggarwal BB. NF- κ B addiction and its role in cancer: 'One size does not fit all'. *Oncogene*. 2010;**30**(14):1615-1630. DOI: 10.1038/onc.2010.566
- [214] Bird L. lncRNA NKILA: A killer regulator. *Nature Reviews Immunology*. 2018;**18**(11):666-667. DOI: 10.1038/s41577-018-0078-3
- [215] Liu B, Sun L, Liu Q, Gong C, Yao Y, Lv X, et al. A cytoplasmic NF- κ B interacting long noncoding RNA blocks I κ B phosphorylation and suppresses breast cancer metastasis. *Cancer Cell*. 2015;**27**(3):370-381. DOI: 10.1016/j.ccell.2015.02.004

- [216] Li X, Shen JK, Hornicek FJ, Xiao T, Duan Z. Noncoding RNA in drug resistant sarcoma. *Oncotarget*. 2017; **8**(40):69086-69104. DOI: 10.18632/oncotarget.19029
- [217] Zhu KP, Ma XL, Zhang CL. LncRNA ODRUL contributes to osteosarcoma progression through the miR-3182/MMP2 axis. *Molecular Therapy*. 2017;**25**(10):2383-2393. DOI: 10.1016/j.ymthe.2017.06.027
- [218] Wang Y, Huang Y, Xiang P, Tian W. LncRNA expression and implication in osteosarcoma: A systematic review and meta-analysis. *OncoTargets and Therapy*. 2017;**10**:5355-5361. DOI: 10.2147/OTT.S149889
- [219] Kong D, Wang Y. Knockdown of lncRNA HULC inhibits proliferation, migration, invasion, and promotes apoptosis by sponging miR-122 in osteosarcoma. *Journal of Cellular Biochemistry*. 2018 Jan;**119**(1): 1050-1061. DOI: 10.1002/jcb.26273
- [220] Cui X, Jing X, Long C, Tian J, Zhu J. Long noncoding RNA MEG3, a potential novel biomarker to predict the clinical outcome of cancer patients: A meta-analysis. *Oncotarget*. 2017;**8**(12): 19049-19056. DOI: 10.18632/oncotarget.14987
- [221] Sun L, Yang C, Xu J, Feng Y, Wang L, Cui T. Long noncoding RNA EWSAT1 promotes osteosarcoma cell growth and metastasis through suppression of MEG3 expression. *DNA and Cell Biology*. 2016;**35**(12):812-818. DOI: 10.1089/dna.2016.3467
- [222] An N, Cheng D. The long noncoding RNA HOST2 promotes gemcitabine resistance in human pancreatic cancer cells. *Pathology and Oncology Research*. 2018;**24**:1-7. DOI: 10.1007/s12253-018-0486-5.
- [223] Huang FX, Chen HJ, Zheng FX, Gao ZY, Sun PF, Peng Q, et al. LncRNA BLACAT1 is involved in chemoresistance of non-small cell lung cancer cells by regulating autophagy. *International Journal of Oncology*. 2018; **54**(1):339-347. DOI: 10.3892/ijo.2018.4614
- [224] Zhu QN, Wang G, Guo Y, et al. LncRNA H19 is a major mediator of doxorubicin chemoresistance in breast cancer cells through a cullin4A-MDR1 pathway. *Oncotarget*. 2017;**8**(54): 91990-92003. DOI: 10.18632/oncotarget.21121
- [225] Chen X, Xie R, Gu P, Huang M, Han J, Dong W, et al. Long noncoding RNA LBCS inhibits self-renewal and chemoresistance of bladder cancer stem cells through epigenetic silencing of SOX2. *Clinical Cancer Research*. 2018. pii: clincanres.1656.2018. DOI: 10.1158/1078-0432.CCR-18-1656
- [226] Wang H, Liu M, Fang L, et al. The cisplatin-induced lncRNA PANDAR dictates the chemoresistance of ovarian cancer via regulating SFRS2-mediated p53 phosphorylation. *Cell Death & Disease*. 2018;**9**(11):1103. Published 2018 Oct 30. DOI: 10.1038/s41419-018-1148-y
- [227] Ding B, Lou W, Xu L, Fan W. Non-coding RNA in drug resistance of hepatocellular carcinoma. *Bioscience Reports*. 2018;**38**(5):BSR20180915. DOI: 10.1042/BSR20180915
- [228] He H, Ni J, Huang J. Molecular mechanisms of chemoresistance in osteosarcoma (Review). *Oncology Letters*. 2014;**7**(5):1352-1362. DOI: 10.3892/ol.2014.1935
- [229] Kun-Peng Z, Xiao-Long M, Chun-Lin Z. LncRNA FENDRR sensitizes doxorubicin-resistance of osteosarcoma cells through down-regulating ABCB1 and ABCC1. *Oncotarget*. 2017;**8**(42): 71881-71893. DOI: 10.18632/oncotarget.17985

- [230] Zhang CL, Zhu KP, Ma XL. Antisense lncRNA FOXC2-AS1 promotes doxorubicin resistance in osteosarcoma by increasing the expression of FOXC2. *Cancer Letters*. 2017;**396**:66-75. DOI: 10.1016/j.canlet.2017.03.018
- [231] Li Z, Zhao L, Wang Q. Overexpression of long non-coding RNA HOTTIP increases chemoresistance of osteosarcoma cell by activating the Wnt/ β -catenin pathway. *American Journal of Translational Research*. 2016;**8**(5): 2385-2393
- [232] Wang Y, Zhang L, Zheng X, Zhong W, Tian X, Yin B, et al. Long non-coding RNA LINC00161 sensitises osteosarcoma cells to cisplatin-induced apoptosis by regulating the miR-645-IFIT2 axis. *Cancer Letters*. 2016;**382**(2): 137-146. DOI: 10.1016/j.canlet.2016.08.024
- [233] Zhang Y, Yang J, Zhao N, et al. Progress in the chemotherapeutic treatment of osteosarcoma. *Oncology Letters*. 2018;**16**(5):6228-6237. DOI: 10.3892/ol.2018.9434
- [234] Hegyi M, Arany A, Semsei AF, et al. Pharmacogenetic analysis of high-dose methotrexate treatment in children with osteosarcoma. *Oncotarget*. 2016;**8**(6):9388-9398. DOI: 10.18632/oncotarget.11543
- [235] Han Z, Shi L. Long non-coding RNA LUCAT1 modulates methotrexate resistance in osteosarcoma via miR-200c/ABCB1 axis. *Biochemical and Biophysical Research Communications*. 2018;**495**(1):947-953. DOI: 10.1016/j.bbrc.2017.11.121
- [236] Zhu KP, Zhang CL, Shen GQ, Zhu ZS. Long noncoding RNA expression profiles of the doxorubicin-resistant human osteosarcoma cell line MG63/DXR and its parental cell line MG63 as ascertained by microarray analysis. *International Journal of Clinical and Experimental Pathology*. 2015;**8**(8): 8754-8773
- [237] Zhang CL, Zhu KP, Shen GQ, Zhu ZS. A long non-coding RNA contributes to doxorubicin resistance of osteosarcoma. *Tumour Biology*. 2016;**37**(2):2737-2748. DOI: 10.1007/s13277-015-4130-7
- [238] Slaby O, Laga R, Sedlacek O. Therapeutic targeting of non-coding RNAs in cancer. *The Biochemical Journal*. 2017 Dec 14;**474**(24): 4219-4251. DOI: 10.1042/BCJ20170079
- [239] Ling H, Fabbri M, Calin GA. MicroRNAs and other non-coding RNAs as targets for anticancer drug development. *Nature Reviews. Drug Discovery*. 2013;**12**(11):847-865. DOI: 10.1038/nrd4140
- [240] Lavorgna G, Vago R, Sarmini M, Montorsi F, Salonia A, Bellone M. Long non-coding RNAs as novel therapeutic targets in cancer. *Pharmacological Research*. 2016;**110**:131-138. DOI: 10.1016/j.phrs.2016.05.018
- [241] Palmieri G, Paliogiannis P, Sini MC, Manca A, Palomba G, Doneddu V, et al. Long non-coding RNA CASC2 in human cancer. *Critical Reviews in Oncology/Hematology*. 2017;**111**: 31-38. DOI: 10.1016/j.critrevonc.2017.01.003
- [242] Ba Z, Gu L, Hao S, Wang X, Cheng Z, Nie G. Downregulation of lncRNA CASC2 facilitates osteosarcoma growth and invasion through miR-181a. *Cell Proliferation*. 2018;**51**(1):e12409. DOI: 10.1111/cpr.12409
- [243] Wang Y, Kong D. LncRNA GAS5 represses osteosarcoma cells growth and metastasis via sponging MiR-203a. *Cellular Physiology and Biochemistry*. 2018;**45**(2):844-855. DOI: 10.1159/000487178

- [244] Ye K, Wang S, Zhang H, Han H, Ma B, Nan W. Long noncoding RNA GAS5 suppresses cell growth and epithelial-mesenchymal transition in osteosarcoma by regulating the miR-221/ARHI pathway. *Journal of Cellular Biochemistry*. 2017;**118**(12):4772-4781. DOI: 10.1002/jcb.26145
- [245] Kirschmann DA, Seftor EA, Hardy KM, Seftor RE, Hendrix MJ. Molecular pathways: Vasculogenic mimicry in tumor cells: Diagnostic and therapeutic implications. *Clinical Cancer Research*. 2012;**18**:2726-2732. DOI: 10.1158/1078-0432.CCR-11-3237
- [246] Zhang Y, Dai Q, Zeng F, Liu H. MALAT1 promotes the proliferation and metastasis of osteosarcoma cells by activating the RAC1/JNK pathway via targeting miR-509. *Oncology Research*. 2018;**26**:1-32. DOI: 10.3727/096504017X14957939026111
- [247] Liu K, Huang J, Ni J, Song D, Ding M, Wang J, et al. MALAT1 promotes osteosarcoma development by regulation of HMGB1 via miR-142-3p and miR-129-5p. *Cell Cycle*. 2017;**16**:578-587. DOI: 10.1080/15384101.2017.1288324
- [248] Yang C, Wang G, Yang J, Wang L. Long noncoding RNA NBAT1 negatively modulates growth and metastasis of osteosarcoma cells through suppression of miR-21. *American Journal of Cancer Research*. 2017;**7**:2009-2019
- [249] Kotake Y, Goto T, Naemura M, Inoue Y, Okamoto H, Tahara K. Long noncoding RNA PANDA positively regulates proliferation of osteosarcoma cells. *Anticancer Research*. 2017;**37**:81-85. DOI: 10.21873/anticancer.11292
- [250] Zou Y, Zhong Y, Wu J, Xiao H, Zhang X, Liao X, et al. Long non-coding PANDAR as a novel biomarker in human cancer: A systematic review. *Cell Proliferation*. 2018;**51**(1):e12422. DOI: 10.1111/cpr.12422
- [251] Zhou Q, Chen F, Zhao J, Li B, Liang Y, Pan W, et al. Long non-coding RNA PVT1 promotes osteosarcoma development by acting as a molecular sponge to regulate miR-195. *Oncotarget*. 2016;**7**(50):82620-82633. DOI: 10.18632/oncotarget.13012
- [252] Song J, Wu X, Liu F, Li M, Sun Y, Wang Y, et al. Long non-coding RNA PVT1 promotes glycolysis and tumor progression by regulating miR-497/HK2 axis in osteosarcoma. *Biochemical and Biophysical Research Communications*. 2017;**490**(2):217-224. DOI: 10.1016/j.bbrc.2017.06.024
- [253] Chen X, Zhou Y, Liu S, Zhang D, Yang X, Zhou Q, et al. LncRNA TP73-AS1 predicts poor prognosis and functions as oncogenic lncRNA in osteosarcoma. *Journal of Cellular Biochemistry*. 2018;**120**(2):2569-2575. DOI: 10.1002/jcb.27556
- [254] Yang G, Song R, Wang L, Wu X. Knockdown of long non-coding RNA TP73-AS1 inhibits osteosarcoma cell proliferation and invasion through sponging miR-142. *Biomedicine & Pharmacotherapy*. 2018;**103**:1238-1245. DOI: 10.1016/j.biopha.2018.04.146
- [255] Bonnetti A, Carninci P. From bench to bedside: The long journey of long non-coding RNAs. *Current Opinion in Systems Biology*. 2017;**33**:119-124. DOI: <https://doi.org/10.1016/j.coisb.2017.04.016>
- [256] Adams BD, Parsons C, Walker L, Zhang WC, Slack FJ. Targeting noncoding RNAs in disease. *The Journal of Clinical Investigation*. 2017;**127**(3):761-771. DOI: 10.1172/JCI84424
- [257] Fatemi RP, Velmeshev D, Faghihi MA. De-repressing lncRNA-targeted genes to upregulate gene expression: Focus on small molecule therapeutics.

Molecular Therapy–Nucleic Acids.
2014;3(11):e196. DOI: 10.1038/
mtna.2014.45

[258] Raimondi L, De Luca A, Costa V,
et al. Circulating biomarkers in
osteosarcoma: New translational tools
for diagnosis and treatment. *Oncotarget*.
2017;8(59):100831-100851. DOI:
10.18632/oncotarget.19852

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