

Long non-coding RNAs: emerging players in osteosarcoma

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Received: 9 December 2015 / Accepted: 27 December 2015 / Published online: 30 December 2015
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Abstract Osteosarcoma is the most common kind of primary bone tumors with high morbidity in infants and adolescents. While the molecular mechanism of osteosarcoma has gained considerable attention, the mechanisms underlying its initiation and progression remain unclear. Recent studies have discovered that long non-coding RNAs (lncRNAs) play an important role in multiply biological processes including cell development, differentiation, proliferation, invasion, and migration. Deregulated expression of lncRNAs has been found in cancers including osteosarcoma. This review summarized the deregulation and functional role of lncRNAs in osteosarcoma and their potential application for diagnosis and treatment of osteosarcoma.

Keywords Osteosarcoma · Long non-coding RNAs · lncRNAs · MALAT1

Introduction

Osteosarcoma is the most common kind of primary bone tumors, which arises from the metaphysis of the long bones

[1–3]. Osteosarcoma occurs in infants and adolescents and the incidence is about 4–5 cases/100,000,000 [4–6]. Despite advancement strategies such as surgery, adjuvant chemotherapy, and radiotherapy, the prognosis of osteosarcoma still remains poor [7–9]. The detail molecular mechanism about metastasis in osteosarcoma patients has not been elucidated [10–12]. Therefore, it is important to find new biomarkers and therapeutic strategies for osteosarcoma management.

Recently, studies have focused on the role of long non-coding RNAs (lncRNAs) in tumor development [13–16]. lncRNAs are a group of non-protein coding transcripts with the length about 200 nucleotides [17, 18]. Increasing studies demonstrated that the lncRNAs play a critical role in many cell processes including cell development, growth, invasion, migration, metastasis, fate decision, and differentiation [19–22]. Moreover, dysregulated expression of lncRNAs has been found in multiple tumors such as gastric cancer, lung cancer, hepatocellular carcinoma, breast cancer, bladder cancer, and ovarian carcinoma [23–29]. Recently, some studies also found that lncRNAs involved in osteosarcoma initiation, growth, and metastasis. In our review, we highlight recent studies regarding the functional role of lncRNAs in osteosarcoma.

Long non-coding RNA expression profiles in osteosarcoma

The first study on lncRNAs expression profiling in osteosarcoma was reported by Li et al. [30] in the 2013. They used lncRNAs expression microarray analysis to detect the different expression of lncRNAs in osteosarcomas tissues and paired adjacent non-tumor tissues. They found that 403 lncRNAs were upregulated and 798 lncRNAs were downregulated in osteosarcomas. Moreover, qRT-PCR was performed to further validate. Their results showed that ASLNC21868,

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ASLNC23844, ASLNC22124, BE503655AS, LNC24587, and BC050642 were upregulated and ASLNC11435, ASLNC00339, ASLNC18814, and ASLNC13387 were downregulated in osteosarcomas.

Further reports on lncRNAs expression profiling in osteosarcomas also identified a number of significantly deregulated lncRNAs, which are listed in Table 1 [31]. They performed the lncRNA-mRNA combined microarray to find the mechanism of doxorubicin chemoresistance of osteosarcomas in terms of lncRNA. They identified 3465 lncRNAs aberrantly expressed in three sets of doxorubicin-resistant MG-63/DXR and their paired MG-63 cells. Among these, 1761 lncRNAs were upregulated and 1704 lncRNAs were downregulated in doxorubicin-resistant MG-63/DXR. Dysregulated lncRNAs were randomly selected to further validate by qRT-PCR. They also found that lncRNA (ENST00000563280 and NR-036444) may play an important role in doxorubicin-resistance of osteosarcomas through interacting with some important genes such as HIF1A, ABCB1, and FOXC2. Moreover, they demonstrated that lncRNA ENST00000563280 was upregulated in specimens of osteosarcomas patients with a poor chemoresponse compared with those with a better chemoresponse. These patients of lower ENST00000563280 expression have a longer survival time than those of higher level.

MALAT1

The metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is also called nuclear-enriched transcript 2 (NEAT2) and mascRNA and contains more than 8700 nucleotides [32–34]. MALAT1 is located on the chromosome 11q13 and was firstly found as a predictive biomarker for metastasis in the early stage of non-small cell lung cancer (NSCLL) [35]. Increasing studies showed that MALAT1 was upregulated in multiply cancers including cervical cancer, endometrial cancer, hepatocellular carcinoma, colorectal cancer, breast cancer, pancreatic cancer, gastric cancer, nasopharyngeal carcinoma, bladder cancer, and oral squamous cell carcinoma [36–43]. Interestingly, the expression of MALAT1 was associated with tumor progression, development, survival, and metastasis in some different tumor types

[32, 44, 45]. MALAT1 can promote cancer cell proliferation, migration, metastasis, and invasion [32, 36].

The first study about the role of MALAT1 in the osteosarcomas was reported by Taniguchi et al. in 2014 [46]. They demonstrated that Myc-6 inhibited malignant phenotypes of osteosarcoma cell line MG-63 cells both in vivo and in vitro. Inhibition of the putative Myc-6 target MALAT1 suppressed the MG-63 cells growth. These data suggested that Myc-6 acts its tumor suppressive role in part through inhibiting MALAT1 expression.

Dong et al. [47] demonstrated that the expression of MALAT1 was upregulated in osteosarcoma tissues and the high expression of MALAT1 was positively corrected with pulmonary metastasis. Inhibition expression of MALAT1 suppressed the osteosarcoma cell proliferation, metastasis, and invasion both in vitro and vivo. Moreover, MALAT1 could suppress the phosphorylated PI3Kp85 α , matrix metalloproteinase 9 (MMP-9), proliferating cell nuclear antigen (PCNA), and Akt expression. These data suggested that MALAT1 played as an oncogenic lncRNA in the development of osteosarcoma.

Fang et al. [48] showed that high dose of 17 β -Estradiol (E2) suppressed the MALAT1 expression in the MG-63 cell and increased the miR-9 expression. Downregulation of MALAT1 increased the combination of SFPQ/PTBP2 complex. Moreover, the inhibition expression of MALAT1 suppressed the MG-63 cell migration, proliferation and invasion, and epithelial-mesenchymal transition (EMT) processes in the E2-dose dependent and estrogen receptor (ER)-independent ways.

Recently, Cai et al. [49] also demonstrated that MALAT1 acts as an oncogenic role in the osteosarcoma. They showed that the expression of MALAT1 was increased in the osteosarcoma tissues and cell lines. Knockdown of MALAT1 suppressed the osteosarcoma cells proliferation and migration and promoted cell cycle arrest and apoptosis. Moreover, inhibition expression of MALAT1 inhibited the tubular network structures formation and caused stress fibers breakage both in MNNG/HOS and U2OS cells. Furthermore, MALAT1 knockdown suppressed tumor growth in vivo and inhibited the RhoA and Rho-associated coiled-coil-containing protein kinases (ROCKs) expression.

Table 1 Long non-coding RNA expression profiles in osteosarcoma

Num	Method	Sample	Upregulated	Downregulated	Reference
1	Microarray RT-PCR	Primary osteosarcoma tissues	403 lncRNAs ASLNC21868, ASLNC23844, ASLNC22124, BE503655AS, LNC24587, BC050642	798 lncRNAs ASLNC11435, ASLNC00339, ASLNC18814, ASLNC13387	[30]
2	Microarray RT-PCR	Doxorubicin Induce-MG-63 cells	1761 lncRNAs ENST00000563280	1704 lncRNAs	[31]

TUG1

TUG1 is a 7.1-kb lncRNA and firstly found in the genomic screen for genes increased in response to taurine application in developing mouse retinal cells [50]. The expression of TUG1 was downregulated in NSCLC and knockdown of TUG1 promoted NSCLC cell proliferation [51]. However, the expression of TUG1 was found to be upregulated in hepatocellular carcinoma, ESCC, and urothelial carcinoma of the bladder [52–54]. TUG1 acts as an oncogene in these tumors.

The first study about the role of TUG1 in the osteosarcomas was reported by Zhang et al. in 2013 [55]. They found that the expression of TUG1 was upregulated in osteosarcoma tissues compared to matched non-tumorous tissues. Moreover, inhibition expression of TUG1 suppressed the osteosarcoma cell proliferation and increased osteosarcoma cell apoptosis. These data supported that TUG1 played an oncogenic lncRNA in the development of osteosarcoma.

In line with previous study, Ma et al. [56] also proved that TUG1 played an oncogenic lncRNA in the development of osteosarcoma. They showed that the expression of TUG1 was upregulated in osteosarcoma tissues and the expression of TUG1 was correlated with post-operative chemotherapy, tumor size, and Enneking surgical stage. The higher expression of TUG1 was associated with poor prognosis and was also a prognostic marker for overall survival (OS).

HIF2PUT

HIF2PUT is also known as TCONS_00004241 and is located at 2p21 on the antisense section of the promoter upstream side of hypoxia-inducible factor-2 α (HIF-2 α) [57]. Wang et al. [57] showed that the expression of HIF2PUT was

downregulated and was positively correlated the expression of HIF-2 α in the osteosarcoma tissues. Upregulation of HIF2PUT suppressed the osteosarcoma cell migration and proliferation and inhibited percentage of CD133 expressing cells. Overexpression of HIF2PUT also could decrease the osteosarcoma stem sphere-forming ability. This result suggested that HIF2PUT acts as a tumor suppressor gene in the osteosarcoma.

H19

H19 is a 2.3 kb of lncRNA molecules and resides close to the telomeric area of human chromosome 11p15.5 [58, 59]. Increasing evidences demonstrated that H19 played an important role in the development and progression of cancers [60, 61]. The expression of H19 was increased in liver, glioblastoma, lung, gastric, and ovarian tumors and increased these cancer cells proliferation and invasion [59, 62–65].

The first study about the role of H19 in the osteosarcomas was reported by Ulaner et al. [66] in 2003. They showed that osteosarcoma cancers with IGF2/H19 maintenance of imprinting (MOI) display allele-specific differential methylation of the CCCTC-binding factor (CTCF)-binding site upstream of H19. Loss of imprinting (LOI) of H19 or IGF2 in the osteosarcoma occurs in the mutually manner and exists with monoallelic expression of other gene. H19 LOI occurs with biallelic hypomethylation of the CTCF-binding site. Moreover, they also demonstrated that aberrant methylation of the IGF2/H19 imprinting control region (ICR) results in equally aberrant CTCF binding. However, the expression of IGF2/H19 is not associated with CTCF binding [67].

Li et al. [68] used microarray analysis to examine global expression changes of the imprinted genes during

Table 2 Functional characterization of the lncRNAs in osteosarcoma

lncRNAs	Expression	Functional role	Related gene	Role	Reference
MALAT1	Up	Proliferation malignant phenotypes migration invasion EMT	Myc-6 PI3Kp85 α MMP-9 PCNA AKT E2	Oncogene	[46–49]
		Apoptosis cell cycle	SFPQ/PTBP2 RhoA ROCKs		
TUG1	Up	Proliferation apoptosis		Oncogene	[55, 56]
HIF2PUT	Down	Proliferation migration CD133	HIF-2 α	Tumor suppressor gene	[57]
H19	LOI Aberrantly		Hh Yap1		[66–69]
Loc285194	CAN depleting			Tumor suppressor gene	[70]

osteosarcoma transformation. Ten imprinted genes such as MKRN3, H19, CDKN1C, NDN, MEST, PHLDA2, GRB10, CD81, SLC22A3, and SLC22A18 were aberrantly regulated in the transformed cells. Moreover, they showed that higher expression of H19 was correlated with the hypomethylation of its promoter regions.

Chan et al. [69] demonstrated that H19 was aberrantly expressed and can be induced through upregulated Hedgehog (Hh) signaling and yes-associated protein 1 (Yap1) overexpression in the osteosarcoma. Their data supported that aberrant expression of Hh signaling in mature osteoblasts is responsible for the osteoblastic osteosarcoma pathogenesis by H19 and Yap1 overexpression.

LOC285194

Loc285194 is also known as LSAMP antisense RNA 3 and consists 4 exons with >2 kbs in length and is located at human chr3q13.31 (osteo3q13.31) [70]. LOC285194 acts a tumor suppressor lncRNA in some tumors. For example, Liu et al. [71] showed that loc285194 was a p53-regulated tumor suppressor lncRNA, which acts its function through inhibition miR-211 expression. Qi et al. [39] demonstrated that the expression of LOC285194 was downregulated in colorectal cancer tissues and cell lines. Low expression of LOC285194 was associated with higher tumor stage, more distant metastasis, and larger tumor size. Ding et al. [40] also demonstrated that the expression of LOC285194 downregulated in human pancreatic ductal adenocarcinoma.

The role of Loc285194 in the osteosarcomas was reported by Pasic et al. in 2010 [70]. They identified a focal region of osteo3q13.31 harboring copy number alteration (CNA) in about 80 % osteosarcomas. Sixty-seven percent of osteo3q13.31 CNAs was deletions with 75 % was monoallelic and accompanied by loss of LOH (heterozygosity) in flanking DNA. Moreover, they showed that these CNAs involve the non-coding RNAs BC040587 and LOC285194. Ubiquitous changes in the BC040587 and LOC285194 in osteosarcoma involving loss of expression. The expression of LOC285194 and BC040587 was associated with the osteo3q13.31 CNAs presence. In addition, depleting LOC285194 increased normal osteoblasts proliferation through regulation of cell cycle and apoptotic transcripts and VEGF receptor 1 expression. The deletions of LOC285194 were correlated with poor survival of osteosarcoma cases (Table 2).

Concluding remarks and future perspectives

Understanding the functional roles of lncRNAs in osteosarcoma represents a crucial way of the current and future study, as lncRNAs may be hallmark features for osteosarcoma. Although there are a lot of difficulties for clinical application

of lncRNA in osteosarcoma diagnosis, development, and treatment, the lncRNA-based study field will keep favorite and encourage investigations for biomarkers and therapeutic targets for osteosarcoma in the future.

Acknowledgments This work was supported by grants from the National Natural Science Foundation of China (NSFC) (Grant Numbers: 81401847, 81272053 and 81330044).

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