Insights into the roles of lncRNAs in skeletal and dental diseases

Yuyu Li1,2, Jiawei Zhang1,2, Jie Pan1,2, Xu Feng3, Peipei Duan1,2, Xing Yin1,2, Yang Xu1,2, Xin Wang1,2 and Shujuan Zou1,2*

Abstract
Long noncoding RNAs (lncRNAs) are a class of non-protein-coding transcripts with the length longer than 200 nucleotides. Growing evidence suggests that lncRNAs, which were initially thought to be merely transcriptional "noise", participate in a wide repertoire of biological processes. It has been well established that lncRNAs not only play important roles in genomic regulation, transcription, posttranscriptional processes but are also implicated in the pathogenesis of human diseases including cardiovascular diseases, diabetes, neurodegenerative disorders, and cancer. However, the pathological role of lncRNAs in skeletal and dental diseases is just beginning to be uncovered. In the present review, we outline the current understanding of the established functions and underlying mechanisms of lncRNAs in various cellular processes. Furthermore, we discuss new findings on the role of lncRNAs in osteoblastogenesis and osteoclastogenesis as well as their involvement in skeletal and dental diseases. This review intends to provide a general framework for the actions of lncRNAs and highlight the emerging evidence for the functions of lncRNAs in skeletal and dental diseases.

Keywords: lncRNA, Osteoblastogenesis, Osteoclastogenesis, Skeletal and dental diseases

Background
The term noncoding RNA (ncRNA) refers to diverse RNA molecules that do not encode any protein. ncRNAs include infrastructural RNAs such as transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), small nuclear RNAs (snRNAs), and small nucleolar RNAs (snoRNAs) [1]. Increasing evidence indicates that additional regulatory ncRNAs such as lncRNAs exist and play important roles in regulating chromatin architecture/epigenetic memory, transcription, and mRNA splicing, stability, and translation [2].

lncRNAs are among the least understood RNAs despite their pervasive transcription in the genome. The most updated annotation of the human genome (Version 27, GRCh38) identifies 27,908 lncRNA transcripts from 15,778 lncRNA genes (http://www.gencodegenes.org/stats/current.html). Like mRNAs, many polyadenylated lncRNAs are transcribed by RNA polymerase II (Pol II) and are often alternatively spliced into multiple isoforms. But genes coding for lncRNAs also have characteristics distinct from those for mRNAs: lncRNA genes have fewer but longer exons, tend to be expressed at lower levels, and exhibit less-conserved sequences [3]. However, some lncRNAs that are expressed in development- and tissue-specific patterns have highly conserved promoter regions and splice sites [4].

lncRNAs do not possess any apparent protein-coding potential and are mostly expressed at low levels; they are thus characterized largely by bioinformatic approaches. Advances in high-throughput RNA sequencing technologies provide systems with which RNA transcription can be observed in an unbiased manner [5]. lncRNAs have been shown to regulate various biological processes via distinct mechanisms [6], whereas mutations or aberrant expression of lncRNAs have been implicated in the pathogenesis of a wide range of human diseases [7–9].
In this review, we briefly present the current knowledge of the functions and mechanisms of lncRNAs. We then review the role of lncRNAs in osteoblastogenesis and osteoclastogenesis based on data from multiple studies. Finally, we discuss the important roles of lncRNAs in the etiology of skeletal and dental disorders.

Functions and mechanisms of lncRNAs
The discovery of the myriad roles of lncRNAs has made it increasingly clear that lncRNAs can function via numerous paradigms and are key regulatory molecules in cells [6]. They not only participate in nuclear events such as chromatin modification and transcription [10], but also reside in the cytoplasm, where they interact with RNA-binding proteins or modulate mRNA translation. Here, we outline a concise scheme of the functions of lncRNAs for a better understanding of what roles lncRNAs play in osteoblastogenesis, osteoclastogenesis, as well as skeletal and dental diseases.

lncRNAs in chromatin modification
In the nucleus, lncRNAs target some chromatin remodeling complexes and guide them to specific genomic loci, leading to changes in gene transcription. A classic example is a long intergenic noncoding RNA (lincRNA) termed X inactive-specific transcript (XIST), which is transcribed from one of the two X chromosomes in female mammals (Fig. 1a). It recruits polycomb group complexes, such as polycomb repressive complex 2 (PRC2) [11], to the female X chromosome, leading to transcriptional silencing in cis across a majority of the chromosome. In contrast, lncRNA HOTAIR, which is transcribed from the antisense strand of homeobox C (HOXC) locus, recruits PRC2 in trans to the HOXD cluster for epigenetic repression [12].

lncRNAs also associate with other chromatin regulators. An example is the lineage-specific imprinting mediated by the lncRNA Kcnq1ot1, a nuclear and moderately stable transcript from the paternal chromosome. In addition to interacting with members of the PRC2 complex, Kcnq1ot1 also recruits chromatin regulators such as G9a methyltransferase to mediate repressive histone modifications, including the trimethylation of lysine 27 on histone H3 (H3K27me3) and trimethylation of lysine 9 on histone H3 (H3K9me3) in the Kcnq1 domain [13].

However, some lncRNAs function in chromatin activation rather than chromatin silencing. Enhancers are regulatory elements that increase the expression of target genes [14]. An enhancer-like lncRNA termed HOTTIP has been identified as a key intermediate that transmits information from higher order chromosomal looping into chromatin modifications. HOTTIP is transcribed from the distal 5′tip of the HOXA locus and is brought into close proximity to multiple HOXA genes by chromosomal looping of the HOXA 5′end. It directly binds the adaptor protein WDR5 and targets WDR5/MLL complexes, inducing H3K4me3 (presented as a pink pentagon with “Me”) and transcription of 5′HOXA genes. Evf-2 as an example of lncRNAs that facilitate transcriptional activation. Combination of Evf-2 with the protein Dlx-2 forms an Evf-2/Dlx-2 complex, which targets the Dlx-5/6 enhancer region and promotes transcription. Question marks indicate that the specific role of Evf-2 in the process remains to be elucidated. d Alu ncRNA can act as a potent transcriptional repressor. Alu RNA contains RNA polymerase II (Pol II, enzyme that synthesizes mRNAs in eukaryotes) binding arms and modular repression domains, allowing it to bind Pol II and block RNA synthesis.
**IncRNAs in transcription regulation**

As shown above, IncRNAs indirectly influence transcription through chromatin modification. However, some IncRNAs regulate transcription directly. The 3.8-kb IncRNA *Evf-2* is transcribed from the *Dlx-5/6* ultraconserved region. *Evf-2* has been found to activate the transcriptional activity of the *Dlx-5/6* enhancer by cooperating with a homeodomain protein Dlx-2 (Fig. 1c). This single-stranded RNA and the ultraconserved protein form an *Evf-2*/Dlx-2 complex and then the complex targets the *Dlx-5/6* enhancer. But whether the complex helps Dlx-2 bind to the enhancer site or only helps stabilize the protein requires further investigation [16]. Nevertheless, some IncRNAs serve as transcriptional repressors [6]. In response to heat shock, the *Alu* ncRNA binds Pol II and enters complexes at promoters, ultimately blocking all detectable RNA synthesis (Fig. 1d). An interesting thing is that although there are sequence discrepancies between the Pol II-binding domains of *Alu* RNA and B2 RNA (*Alu* RNA-like sequences in mouse), both of them repress transcription by Pol II. So the mechanism by which *Alu* RNA functions may not be sequence specific [17].

**IncRNAs in pre-mRNA splicing**

In the nucleus, IncRNAs are implicated in posttranscriptional regulatory steps, including pre-mRNA splicing, mRNA capping, polyadenylation, and nuclear export. Pre-mRNA splicing is a key process to increase proteome diversity in higher eukaryotes [18]. The IncRNA metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) has been proposed to regulate alternative splicing (AS) by modulating the distribution of active serine/arginine-rich (SR) proteins in nuclear speckle domains. SR proteins are essential splicing factors that function in both constitutive splicing and AS [19, 20]. Previous studies indicated that *MALAT1* modulated AS of endogenous pre-mRNAs by regulating SR splicing factors phosphorylation, as well as altering the distribution and ratio of phosphorylated versus non-phosphorylated pools of SR proteins (Fig. 2a). These changes may lead to alterations in the expression of specific isoforms of proteins in cells [21].

Another example of involvement of IncRNA in AS is *sno-IncRNA*, a class of nuclear-enriched intron-derived IncRNAs transcribed from a critical region of chromosome 15 (15q11-q13). This region is specifically deleted in Prader–Willi Syndrome (PWS). *sno-IncRNAs* are flanked by snoRNA sequences at both ends [22]. Studies have demonstrated that at least some of these *sno-IncRNAs* acted as molecular sinks of the splicing regulator Fox2, a member of the Fox family. *sno-IncRNAs* binded to Fox2 and altered the splicing patterns. Importantly, *sno-IncRNA* knockdown led to changes in Fox2-regulated splicing, while the overall gene expression levels were unaltered [23].

**IncRNAs in mRNA protection and decay**

As an intermediate, mRNA carries information from genes to ribosomes for protein synthesis. However, it is
unstable and the concentration of mRNA depends on the balance between the rates of synthesis and degradation [24]. lncRNAs are increasingly recognized as important modulators of both mRNA synthesis and degradation.

Functional characterization of the lncRNA BACE1-AS has revealed the action of lncRNAs in maintaining mRNA stability. BACE1-AS is a conserved antisense transcript partner of β-site amyloid precursor protein cleaving enzyme 1 (BACE1), which is a crucial enzyme in the pathogenesis of Alzheimer’s disease. When exposed to amyloid-β 1–42 (Aβ 1–42), which can induce oxidative stress, elevated BACE1-AS levels increase BACE1 mRNA stability and generate additional Aβ 1–42 through a post-transcriptional feed-forward mechanism [25] (Fig. 2b).

Another type of lncRNA exerts its function by facilitating mRNA decay. Staufen 1 (STAU1) is a double-stranded RNA binding protein that binds to a subset of mRNAs and targets them for STAU1-mediated mRNA decay (SMD) [26]. Half-STAU1-binding site RNA (1/2-sbsRNA) is a polyadenylated lncRNA that induces mRNA decay by recruiting STAU1 to target mRNAs. STAU1-binding sites can be formed by imperfect base-pairing between an Alu element of an mRNA target of SMD and another Alu element in 1/2-sbsRNA. The 1/2-sbsRNA-regulated mRNAs such as CUB-domain-containing protein 1 (CDCCPI) mRNA and methylthioadenosine phospholysase (MTAP) mRNAs can thus be degraded [27] (Fig. 2c).

**IncRNAs in translation activation and repression**

mRNA translation is the final step of protein synthesis. Recent studies have demonstrated that some lncRNAs control protein synthesis by either post-transcriptional activation or repression of mRNA translation in the cytoplasm.

The nuclear-enriched lncRNA, antisense Uchl1, forms sense-antisense pairs by pairing with the ubiquitin carboxy-terminal hydrolase L1 (Uchl1) gene. Under stress conditions, antisense Uchl1 lncRNA shuttles from the nucleus to the cytoplasm. It then binds the 5’ end of the Uchl1 mRNA and promotes the association of this overlapping sense protein-coding mRNA with active polysomes for translation [28] (Fig. 2d).

On the contrary, lncRNA-p21 inhibits the translation of target mRNAs encoding β-catenin (CTNNB1) and JUNB (JUNB) after HuR (also known as embryonic lethal abnormal vision 1, ELAVL1) is silenced. HuR is a ubiquitous RNA-binding protein that functions in cell proliferation, survival, and carcinogenesis, as well as in stress and immune responses [29, 30]. HuR exerts its functions mainly by interacting with a subset of mRNAs, and further increasing their stability and modulating their translation [31]. HuR also enhances the decay of lncRNA-p21. Therefore, in the absence of HuR, stable lncRNA-p21 inhibits the translation of CTNNB1 and JUNB mRNAs by enhancing their interaction with the translational repressors Rck, which may result in polysome size reduction and even ribosome “drop-off” [32] (Fig. 2e).

**IncRNAs in miRNA biology**

microRNAs (miRNAs) are endogenous 19–23-nucleotide RNAs that negatively regulate gene expression at the post-transcriptional level. They interact with partially complementary sequences in the 3’-UTR of a target mRNA, leading to translational repression, mRNA cleavage, and mRNA decay [33, 34]. Recent reports have demonstrated that lncRNAs may prevent the repressive effects of miRNAs on their targets [35–37]. lncRNAs function as competing endogenous RNAs (ceRNAs) to sequester miRNAs, thereby protecting the target mRNAs from degradation [38].

*linc-MD1* has been implicated as a ceRNA that competes for shared miRNAs with mRNAs. Therefore, it can be regarded as an activator in mRNA translation. *Linc-MD1* “sponges” miR-133 and miR-135 to regulate the mRNA translation of mastermind-like-1 (MAML1) and myocyte-specific enhancer factor 2C (MEF2C), respectively (Fig. 2f). With the finding that both MAML1 and MEF2C are critical genes for normal myogenic differentiation [39], *linc-MD1* is postulated to be involved in the control of myoblast differentiation [40]. The well-known lncRNA *H19* has been identified as a novel activator of the Wnt/β-catenin pathway by serving as a miRNA sponge. *H19* antagonizes the functions of miR-141 and miR-22, both of which are negative modulators of the Wnt/β-catenin pathway and osteogenesis. The presence of *H19* leads to the derepression of their shared target gene, β-catenin, and eventually promotes osteoblast differentiation [41].

**Modulation of osteoblastogenesis and osteoclastogenesis by IncRNAs**

Mesenchymal stem cells (MSCs) have the potential to differentiate into multiple cell types, including osteoblasts, chondrocytes, adipocytes, and neurocytes [42]. Osteoclasts are derived from hematopoietic precursor cells of the monocyte-macrophage lineage. They are large, multinucleated, terminally differentiated cells, functioning as the sole bone-resorbing cells [43]. Skeletal development and adult bone remodeling depend on the coordinated function of osteoblasts and osteoclasts, which differentiate from precursor cells in the mesenchymal osteoblastic lineage [44] and the hematopoietic osteoclastic lineage [45], respectively.

The involvement of lncRNAs in the differentiation of MSCs into osteoblasts has been unveiled over the past
decade (Table 1). Analysis of IncRNA expression profiles has revealed significant differences between untreated and bone morphogenetic protein 2 (BMP-2)-treated C3H10T1/2 MSCs [46]. In the study, the authors used BMP-2 to induce early osteoblastogenesis, and compared the differential expression profiles of IncRNA by microarray and bioinformatic approaches. Over 100 differentially expressed IncRNAs were identified. A subset of 24 IncRNAs was determined to concurrently change with their nearby coding genes, which are involved in osteoblastogenesis. For example, mouselincRNA0231 and its nearby gene epidermal growth factor receptor (EGFR), which suppressed osteoblast differentiation via regulating Runx2 and Osterix, were downregulated after BMP-2 treatment. A similar correlation was observed between NR_027652 and mouselincRNA0243 with their respective nearby coding genes DLK1 and IL-5, respectively. Another study demonstrated that anti-differentiation ncRNA (ANCR) regulated Runx2 expression and osteoblastogenesis. ANCR interacted with the enhancer of zeste homolog 2 (EZH2). The recruitment of ANCR with EZH2 catalyzed H3K27me3 in Runx2 gene promoter, resulting in the inhibition of Runx2 expression and subsequent osteoblastogenesis [47].

The function of the IncRNA hypoxia-inducible factor 1α-anti-sense 1 (HIF1a-ASI) in osteoblastogenesis was recently identified. HIF1α-ASI expression was significantly repressed after overexpression of the histone deacetylase sirtuin 1 (SIRT1), an important regulator of osteoblast differentiation [48]. Lower levels of SIRT1 gave rise to the upregulation of HIF1α-ASI in human bone marrow stem cells (BMSCs). Moreover, HIF1α-ASI knockout inhibited the expression of HOXD10 by interfering with acetylation, suggesting the potential role of HIF1α-ASI in the activation of osteoblastogenesis [49].

Attention has also been paid to the effects of IncRNAs on dentinogenesis (Table 1). Dentinogenesis shares many similarities with osteogenesis, and consists of multiple steps including odontoblast differentiation. Chen et al. showed that IncRNAs were involved in the odontoblast-like differentiation of human dental pulp cells (hDPCs) [50]. In their study, the expression of the differentiation-antagonizing IncRNA DANCR was considerably downregulated in a time-dependent manner in the process of hDPCs differentiation into odontoblast-like cells. Furthermore, mineralized nodule formation as well as the expression of dentin sialophosphoprotein and dentin matrix protein-1 was blocked after overexpression of DANCR in hDPCs. Upregulation of DANCR also decreased the expression levels of p-GSK-3β and β-catenin. These results reveal a role of DANCR in regulating the Wnt/β-catenin pathway and modulating dentin formation.

IncRNAs also play a regulatory role in osteoclastogenesis (Table 1). In one study, microarray analysis was performed to examine the expression profiles of IncRNAs at different stages of osteoclastogenesis. Then gene ontology analysis, pathway analysis, and IncRNA-mRNA co-expression network characterization showed the co-expression of multiple IncRNAs with tumor necrosis factor ligand superfamily member (TNFSP12) and TNFSP13 [51], factors involved in the differentiation of monocyte/macrophage precursor cells into osteoclasts [52, 53]. Circulating monocytes are directly involved in osteoclastogenesis by acting as osteoclast precursors [54]. The role of IncRNA DANCR in blood mononuclear cells has been
studied. Overexpression of DANCR increased the secretion of IL-6 and TNF-α in blood mononuclear cells [55], both of which were inflammatory cytokines and important mediators of accelerated bone loss in osteoporosis [56, 57]. This suggests that DANCR can be a potential biomarker and regulatory element in circulating monocytes for osteoclastogenesis. However, further studies are needed to determine the underlying mechanisms of IncRNAs in osteoclastogenesis.

**IncRNAs in skeletal and dental diseases**

IncRNAs not only play critical roles in various aspects of cellular biology but are also implicated in disease pathogenesis and progression. Several IncRNAs have been functionally associated with important pathogenic processes of cardiovascular diseases [58], diabetes [59], neurodegenerative disorders [60], immune response [61], as well as several types of cancer [62, 63]. However, the identity of IncRNAs in skeletal and dental diseases is not well known. Here, we summarize new findings in the functions of IncRNAs in these diseases (Table 1).

**Osteoporosis**

Emerging evidence demonstrates the correlation of IncRNAs with osteoporosis, which is a common metabolic bone disorder [55]. Osteoporosis is characterized by reduced bone mineral density and increased incidence of fractures, resulting mainly from enhanced osteoclastic bone resorption activity outpacing bone formation by osteoblasts [64]. The sequence encoding IncRNA DANCR resides on human chromosome 4, located 54.8 kb upstream of USP46 and 28.7 kb downstream from ERVMER34-1 and the ANCR locus. As mentioned above, DANCR was upregulated in circulating monocytes of postmenopausal women with low bone mineral density, and could induce the expression of IL-6 and TNF-α [55]. These results suggest the important role of DANCR in the pathogenesis of osteoporosis and possibly as a biomarker for postmenopausal osteoporosis (PMOP). Another case is the involvement of IncRNA MEG3 in the pathogenesis of PMOP [65]. In this study, MEG3 expression was increased in BMSCs derived from PMOP patients and ovariectomized mice. MEG3 directly bound to and activated miR-133a-3p, thereby inhibiting the expression of SLC39A1 (a direct target of miR-133a-3p), which was regarded as a positive regulator of osteogenic differentiation. Overexpression of MEG3 inhibited osteogenic differentiation of BMSCs, which was markedly reversed by miR-133a-3p knockdown. These data indicate that IncRNAs participate in the pathogenesis of osteoporosis, which provide novel targets for the prevention and treatment of osteoporosis.

**Osteoarthritis**

Osteoarthritis (OA) is the clinical and pathological outcome of a range of disorders that results in structural and functional failure of synovial joints. While many risk factors (e.g., IL-1, IL-6, TNF-α, PGE2, MMPs) contribute to the onset of OA [69], the mechanism responsible for OA has not been fully elucidated. Recent studies have investigated the effects of IncRNAs on OA. Xing et al. reported that over 100 IncRNAs were up- or down-regulated in OA cartilage compared with normal cartilage based on microarray analysis. The increased expression of six IncRNAs (HOTAIR, GASS, PMS2L2, RP11-445H22.4, H19 and CTD-2574D22.4) in the microarray data was validated by real-time PCR [70], suggesting the regulatory potential of IncRNAs in OA. SOX9 nc 2 is a cartilage-specific IncRNA which lies upstream of SOX9 in the genome. Depletion of the SOX9 nc 2 transcript by RNA interference prevented chondrogenesis and the expression of the transcription factor SOX9 [71]. Moreover, a significant correlation has been observed among the expression of IncRNA H19, miR-675, and COL2A1 in OA cartilage. Co-upregulation of H19, COL2A1, and miRNA-675 was observed in chondrocytes under hypoxic conditions, which were known to stimulate chondrocyte anabolism. When chondrocytes were treated with inflammatory factors IL-1β and TNF-α to induce chondrocyte catabolism, the expression of H19, COL2A1, and miRNA-675 was significantly decreased [72]. In addition, Dudek et al. showed that inhibition of H19 downregulated COL2A1, while overexpression of

**Skeletal transformation**

After its first identification in primary human fibroblasts [66], the IncRNA Hotair has also been found to be important in the embryonic patterning of the skeletal system [67]. Targeted deletion of Hotair resulted in lumbosacral homeotic transformation (6th lumbar vertebrae transform to 1th sacral vertebrae, L6 → S1) in a C57BL/6 mouse model. Moreover, malformation of the metacarpals and 4th caudal vertebrae was also observed. Hotair knockdown caused derepression of multiple HoxD cluster genes in embryos and tail tip fibroblasts. Insights into the molecular basis for the observed phenotypes revealed that Hotair acted in trans to bind both PRC2 and LSD1 complex. Hotair recruited them to hundreds of genomic sites to promote coordinated H3K27 methylation and H3K4 demethylation for gene silencing. However, in another study, the skeletal malformation indicated above was not detected after Hotair knockdown in a mixed CBAxBL/6 mouse model [68]. Whether the discrepancy in the phenotypic effects of Hotair knockdown is attributed to the different genetic background of animals needs further study.
miR-675 rescued COL2A1 expression in H19-depleted human articular chondrocytes [73]. More work is needed to investigate the function and mechanisms of lncRNAs as key regulators of OA.

Periodontitis
Evidence for the relationship between periodontitis and lncRNAs is emerging. Periodontitis is a common chronic inflammatory disease initiated by a group of bacterial pathogens in dental plaque. The inflammation extends deep into tissues, damages the connective tissue and alveolar bone around teeth, and eventually leads to tooth loss [74]. Microarray analysis of lncRNA expression profile revealed a total of 8925 differently expressed lncRNAs in chronic periodontitis tissues compared with adjacent normal tissues. Further subgroup analysis showed there were 589 enhancer-like lncRNAs, 238 HOX cluster lncRNAs, as well as 1218 lincRNAs. Based on the information, the function and mechanisms of lncRNAs associated with periodontitis needs further investigation [75]. The role of a crucial lncRNA related to periodontitis, IncRNA-POIR, has recently been investigated. lncRNA-POIR expression was significantly lower in periodontal mesenchymal stem cells (PDLSCs) from periodontitis patients (pPDLSCs) than that in human periodontal MSCs (hPDLSCs). Overexpression of IncRNA-POIR promoted osteogenic differentiation of pPDLSCs. Further study revealed that IncRNA-POIR acted as a ceRNA for miR-182, thus positively regulating expression of FoxO1. The inflammatory environment, which usually occurred in periodontitis, increased miR-182 expression through NF-κB pathway, finally resulted in an imbalance in the IncRNA-POIR-miR-182 regulatory network [76]. The association between periodontitis and another lncRNA ANRIL has also been reported. ANRIL is the first shared genetic risk factor of coronary artery disease and aggressive periodontitis [77]. Bochenek et al. demonstrated that ANRIL knockdown resulted in repression of three genes ADIPOR1, VAMP3, and C11ORF10. Exploration of the identified genes highlighted a region upstream of VAMP3 within CAMTA1 (rs10864294) to be associated with increased risk of coronary artery disease and aggressive periodontitis [78]. These studies indicate the potential of lncRNAs as diagnostic biomarkers and targets for the treatment of periodontitis.

Osteosarcoma
Differences in the expression of lncRNAs in different types of tumors have been well documented [79, 80]. This finding promoted interest in addressing the potential of lncRNAs in skeletal tumors. Osteosarcoma is the most common primary malignant tumor of bone with cytogenetic complexity. The lncRNA TUSC7 (tumor suppressor candidate 7), previously named as LOC285194, was significantly downregulated in osteosarcomas. The decreased expression of TUSC7 was due to copy number loss of the genomic region on chr3q13.31. Depletion of TUSC7 promoted proliferation and inhibited apoptosis in osteosarcoma cells. TUSC7 suppression also increased osteosarcoma growth in a mouse model, and was correlated with poor survival of osteosarcoma patients [81, 82]. In addition, recent studies revealed that the lncRNA MALAT1 was dysregulated in multiple malignant tumors, including osteosarcoma. Knockdown of MALAT1 decreased proliferation, migration, and induced apoptosis in osteosarcoma. MALAT1 knockdown significantly inhibited PI3K/AKT and RhoA/ROCK signaling pathway. High expression of MALAT1 was closely correlated with pulmonary metastasis in patients with osteosarcoma [83, 84]. Interestingly, Fang et al. demonstrated that downregulation of MALAT1 induced by high dose of 17β-Estradiol promoted the binding of SFPQ to oncogene PTBP2, therefore affecting proliferation, migration or invasion in osteosarcoma cells [85]. Further discussion of lncRNAs in osteosarcoma can be found elsewhere [86, 87].

Ameloblastoma
Ameloblastoma is a benign but locally invasive odontogenic tumor of the jaws [88]. It often results in facial deformity and significant morbidity because of its high rate of recurrence and requirement for radical surgery. Considerable efforts have been made to clarify the underlying molecular mechanisms and actions of lncRNAs in ameloblastoma. The ncRNA expression profile of ameloblastoma was characterized in a well-defined ameloblastoma cohort. In this study, whole transcriptome profiling by microarray followed by real-time PCR assays validated five highly associated ncRNAs, including the lncRNA LINC340 (also known as CASC15). However, whether LINC340 is a prognostic and therapeutic marker that can improve the treatment of ameloblastoma requires further investigation [89].

Conclusions
Over the past decade, extensive research has established that lncRNAs play important roles in diverse cellular processes. Moreover, the molecular mechanisms by which lncRNAs exert their functions have been largely elucidated. These discoveries have promoted investigators in the skeletal and dental fields to address the potential role of lncRNAs in regulating the differentiation and function of bone cells as well as in the pathogenesis of skeletal and dental diseases. However, whereas a few studies have revealed the functional role of some lncRNAs, most of the results have merely
demonstrated an association of lncRNAs with either bone cell biology or the development of some skeletal and dental diseases. Hence, future investigations should focus on further establishing the functional links between lncRNAs and hard tissue diseases and elucidating the underlying molecular mechanisms. A better understanding of the regulatory roles and molecular mechanisms of lncRNAs in skeletal and dental diseases may identify new biomarkers for diagnosis and novel therapeutic targets for these disorders.

Abbreviations
ANCR: anti-differentiation noncoding RNA; AS: alternative splicing; AB 1-42: amyloid-b 1-42; BACE1: β-secretase-1; BMSC: bone marrow stem cell; BMP-2: bone morphogenetic protein 2; CDCA1: CUB-domain-containing protein 1; ceRNA: competing endogenous RNA; DANCR: differentiation-antagonizing long noncoding RNA; EGF: epidermal growth factor receptor; EZH2: enhancer of zeste homolog 2; HDPC: human dental pulp cell; HIF1α-A51: hypoxia-inducible factor 1α-anti-sense 1; HOX: homeobox; hPDLCSC: human periodontal mesenchymal stem cell; H3K4me3: trimethylation of lysine 4 on histone H3; H3K9me3: trimethylation of lysine 9 on histone H3; H3K27me3: trimethylation of lysine 27 on histone H3; lncRNA: long intergenic noncoding RNA; IncRNA: long noncoding RNA; LSD1: lysine-specific demethylase 1; MALAT1: metastasis-associated lung adenocarcinoma transcript 1; MAML1: mastermind-like-1; MEF2C: myocyte-specific enhancer factor 2C; miRNA: microRNA; MSC: mesenchymal stem cell; MTAP: methylthioadenosine phosphorylase; ncRNA: non-protein-coding RNA; OA: osteoarthritis; PDLSC: periodontal mesenchymal stem cell; PFO: postmenopausal osteoporosis; Pol II: polymerase II; PPDLCSC: patient periodontal mesenchymal stem cell; PRC2: polycomb repressive complex 2; PWS: Prader–Willi Syndrome; rRNA: ribosomal RNA; RT-PCR: real-time polymerase chain reaction; SIRT1: sirtuin 1; SMD: STAU1-mediated mRNA decay; snRNA: small nuclear RNA; spRNA: small nuclear RNA; SR protein: serine/arginine-rich protein; STAU1: Staufen 1; TGF: transforming growth factor; tRNA: transfer RNA; Uchl1: ubiquitin carboxy-terminal hydrolase L1; XIST: X inactive-specific transcript; 1/2-sbsRNA: half-STAU-1-binding site RNA; 3'UTR: 3' untranslated region.

Authors' contributions
YL wrote the manuscript. JZ, JP and PD created the figures. XY, YX and YW made the table and revised the manuscript. XF and SZ revised and approved the manuscript. JZ, JP and PD created the figures. XY, YX and YW made the table and revised the manuscript. XF and SZ revised and approved the manuscript. YL wrote the manuscript. JZ, JP and PD created the figures. XY, YX and YW made the table and revised the manuscript. XF and SZ revised and approved the manuscript. MZ, ER and ZWJY made the table and revised the manuscript. XF and SZ revised and approved the manuscript. SNJ made the table and revised the manuscript. XF and SZ revised and approved the manuscript. JZ, JP and PD created the figures. XY, YX and YW made the table and revised the manuscript. XF and SZ revised and approved the manuscript.

Authors details
1 State Key Laboratory of Oral Diseases, National Clinical Research Center for Oral Diseases, West China Hospital of Stomatology, Sichuan University, No.14, 3rd Section, Renmin South Road, Chengdu 610041, China. 2 Department of Orthodontics, West China Hospital of Stomatology, Sichuan University, No.14, 3rd Section, Renmin South Road, Chengdu 610041, China. 3 Department of Pathology, University of Alabama at Birmingham, 1670 University Blvd., VH G019E, Birmingham, AL 35294, USA.

Acknowledgements
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
Not applicable.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Not applicable.

Funding
This work was supported by the National Natural Science Foundation of China [Grant Number: 81470777].

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 16 August 2017 Accepted: 30 January 2018
Published online: 05 February 2018

Li et al. Cell Biosci (2018) 8:8

References
1. Mattick JS, Makunin IV. Non-coding RNA. Hum Mol Genet. 2006;15:R17–29.
2. Schmitz SJ, Grote P, Herrmann BG. Mechanisms of long noncoding RNA function in development and disease. Cell Mol Life Sci. 2016;73:2491–509.
3. Demien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Mikel A, Knowles DG, et al. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res. 2012;22:1775–89.
4. Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell. 2009;136:629–41.
5. Lee C, Kikyo N. Strategies to identify long noncoding RNAs involved in gene regulation. Cell Biosci. 2012;2:37.
6. Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. Genes Dev. 2009;23:1494–504.
7. Szymanski M, Barczewska MZ, Erdmann VA, Barczewski J. A new frontier for molecular medicine: noncoding RNAs. BBA-Rev Cancer. 2005;1756:65–75.
8. Prasanth KV, Spector DL. Eukaryotic regulatory RNAs: an answer to the ‘genome Complexity’ conundrum. Genes Dev. 2007;21:11–42.
9. Taft RJ, Pang KC, Mercer TR, Dinger M, Mattick JS. Non-coding RNAs: regulators of disease. J Pathol. 2010;221:26–39.
10. Chen L-L, Garmichael GC. Decoding the function of nuclear long noncoding RNAs. Curr Opin Cell Biol. 2010;22:357–64.
11. Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. Nature. 2011;469:343–9.
12. Rinn JL. lncRNAs: linking RNA to chromatin. Cold Spring Harb Perspect Biol. 2011;6:a019331.
13. Pandey RR, Mondal T, Mohammad F, Enroth S, Redrup L, Komorowski J, Nagano T, Mancini-DiNardo D, Kanduri C. Kcnq1ot1 antisense ncoding RNA mediates lineage-specific transcriptional silencing through chromatin-remodelling regulation. Mol Cell. 2008;32:232–46.
14. Lam MTY, Li W, Rosenfeld MG, Glass CK. Enhancer RNAs and regulated transcriptional programs. Trends Biochem Sci. 2014;39:170–82.
15. Wang KC, Yang YW, Liu B, Sanyal A, Corces-Zimmerman R, Chen Y, Lajoie BR, Ptatiao A, Flynn RA, Gupta RA, et al. A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. Nature. 2011;472:120–4.
16. Feng J, Bi C, Clark BS, Mady R, Shah P, Kohtz JD. The Evf-2 noncoding RNA is transcribed from the Dlx-5/6 ultraconserved region and functions as a Dlx-2 transcriptional coactivator. Genes Dev. 2006;20:1470–84.
17. Marinier PD, Walters RD, Esponza CA, Durringer LF, Wagner SD, Kugel JF, Goodrich JA. Human Alu RNA is a modular transacting repressor of mRNA transcription during heat shock. Mol Cell. 2008;29:499–509.
18. Hallekger M, Llorian M, Smith CWJ. Alternative splicing: global insights. FEBS J. 2010;277:856–66.
19. Fu X-D, Ares MJr. Context-dependent control of alternative splicing by RNA-binding proteins. Nat Rev Genet. 2014;15:689–701.
20. Reddy ASN. Plant serine/arginine-rich proteins and their role in pre-mRNA splicing. Trends Plant Sci. 2004;9:541–7.
21. Tripathi V, Ellis JD, Shen Z, Song DY, Pan Q, Watt AT, Freier SM, Bennett CF, Sharma A, Bubulya PA, et al. The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. Mol Cell. 2010;39:925–38.
22. Reaick D, Prakash A, McSweeney A, Shepard SS, Fedorova L, Fedorov A. Critical association of ncRNA with introns. Nucleic Acids Res. 2011;39:2357–66.
23. Yin Q-F, Yang L, Zhang Y, Xiang J-F, Wu Y-W, Carmichael Gordon G, Chen L-L. Long noncoding RNAs with snorRNA ends. Mol Cell. 2012;48:219–30.

24. Pérez-Ortín JE, Alepuz P, Chávez S, Choder M, Eukaryotic mRNA decay: methodologies, pathways, and links to other stages of gene expression. J Mol Biol. 2013;425:750–75.

25. Faghihi MA, Modaresi F, Khalil AM, Wood DE, Sahagan BG, Morgan TE, Finch CE, St. Laurent ii-G, Kenny PJ, Wahlestedt C. Expression of a noncoding RNA is elevated in Alzheimer's disease and drives rapid feed-forward regulation of [beta]-secretase. Nat Med. 2008;14:723–30.

26. Kim YK, Furic L, Pslisn M, Major F, DesGrosselliers L, Maquat LE. Staufen1 regulates diverse classes of mammalian transcripts. EMBO J. 2007;26:2670–81.

27. Gong C, Maquat LE. IncRNAs transactivate STAU1-mediated mRNA decay by duplexing with 3′[pimel] UTRs via Aliu elements. Nature. 2011;470:284–8.

28. Carriere C, Cimmati L, Bagnoli M, Beugnet A, Zuccelli S, Fedele S, Pesce E, Ferrer I, Collavi S., Sancovito C, et al. Long non-coding antisense RNA controls Uchl1 translation through an embedded SINEB2 repeat. Nature. 2012;491:454–7.

29. Abdelmohsen K, Gorospe M. Posttranscriptional regulation of cancer traits by HuR. WIREs RNA. 2010;1:214–29.

30. Hinman MN, Lou H. Diverse molecular functions of Hu proteins. Cell Mol Biol. 2009;55:3168.

31. Mukherjee N, Corcoran David L, Nusbbaum J, David Reid W, Geor- giev S, Hafner M, Ascano M Jr, Tsutch T, Ohler U, Keene J. Integrative regulatory mapping indicates that the RNA-binding protein HuR couples pre-mRNA processing and mRNA stability. Mol Cell. 2011;43:327–39.

32. Yoon JH, Abdelmohsen K, Sirkantian S, Yang X, Martindale Jennifer L, De S, Huarte M, Zhan M, Becker Kevin G, Gorospe M. LincRNA-p21 sup- presses target mRNA translation. Mol Cell. 2012;47:648–55.

33. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009;136:215–33.

34. Baed D, Villen J, Shin C, Camargo FD, Gygi SP, Bartel DP. The impact of microRNAs on protein output. Nature. 2008;455:64–71.

35. Deng L, Yang S-B, Xu F-F, Zhang J-H. Long noncoding RNA CCAT1 promotes hepatocellular carcinoma progression by functioning as let-7 sponge. J Exp Clin Curr Res. 2013;34:18.

36. Xing C, Hu X, Xie F, Yu Z, Li H, Jin Z, Wu J, Tang L, Gao S. Long non-coding RNA HOTAIR modulates c-Kit expression through sponging miR-193a in acute myeloid leukemia. FEBS Lett. 2015;589:1981–7.

37. Cai H, Xue Y, Wang P, Wang Z, Li Z, Hu Y, Li Z, Shang X, Liu Y. The long noncoding RNA TUG1 regulates diverse classes of mammalian transcripts. EMBO J. 2007;26:2670–81.

38. Yoon J-H, Abdelmohsen K, Gorospe M. Functional interactions among microRNAs and long noncoding RNAs. Semin Cell Dev Biol. 2014;34:9–14.

39. Shen H, McElhinny AS, Cao G, Gao P, Liu J, Bronson R, Griffin JD, Wu L. The notch coactivator, MAML1, functions as a novel coactivator for MEF2C-mediated transcription and is required for normal myogenesis. Genes Dev. 2005;19:675–88.

40. Cesana M, Cacciarelli D, Legnini I, Santini T, Sthandier O, Chinappi M, Tramontano A, Bozzoni I. A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. Cell. 2011;147:358–69.

41. Liang W, Fu W, Wang Y, Sun Y, Xu L, Gong C, Chan K, Li G, Weye MM, Zhang J. H19 activates Wnt signaling and promotes osteoblast differentiation by functioning as a competing endogenous RNA. Sci Rep. 2016;6:20121.

42. Kapinas K, Kessler C, Ricks T, Gronowicz G, Delany AM. miR-29 modulates macrophage polarization by regulating monocyte mRNA expression. BMC Mol Biol. 2009;10:81.

43. Hemingway F, Taylor R, Knowles HJ, Athanasou NA. RANKL-independent human osteoclast formation with APRIL, BAFF, NGF, IGF I and IGF II. Bone. 2011;48:938–46.

44. Fukushima Y, Quinn JM, Sabokbar A, McGee JO, Athanasou NA. The human osteoclast precursor circulates in the monocyte fraction. Endocrinology. 1996;137:4058–60.

45. Kong X, Gu P, Xu S, Lin X. Long non-coding RNA-DANCR in human circulat- ing monocytes: a potential biomarker associated with postmenopausal osteoporosis. Biosci BioTech Bioch. 2015;79:732–7.

46. Yoo B, Chang J, Liu Y, Li J, Kevork K, Al-Hezmari K, Graves DT, Park N, Wang C. Wnt signaling prevents skeletal aging and inflammation by inhibiting nuclear factor-κappaB. Nat Med. 2014;20:1009–17.

47. Pacifici R, Brown C, Puschcheck E, Friedrich E, Slatopolsky E, Maggio D, McCracken R, Avioli LV. Effect of surgical menopause and estrogen replacement on cytokine release from human blood mononuclear cells. Proc Nat Acad Sci. 1991;88:1534–8.

48. Morán I, Akerman I, van de Bunt M, Xie R, Benazra M, Narnmo T, Arnes N, Nakic G, Garcia-Hurtado J, Rodriguez-Segui S, et al. Human β cell transcriptome analysis uncovers IncRNAs that are tissue-specific, dynami- cally regulated, and aberrantly expressed in type 2 diabetes. Cell Metab. 2012;16:435–48.

49. Salta E, De Strooper B. Non-coding RNAs with essential roles in neurode- generative disorders. Lancet Neurol. 2012;11:189–200.

50. Huang DB, Carranza MA. Non-coding RNAs in the regulation of the human immune response. Trends Immunol. 2014;35:408–19.

51. Yang G, Lu X, Yuan L. LncRNA: a link between RNA and cancer. BBA-Gene Regulation and Signaling. 2015;1858:1097–109.

52. Park J, Kwok S, Lim M, Oh H, Kim J, Jhun J, Ju JH, Park K, Park Y, Park S, et al. TWEAK promotes osteoclastogenesis in rheumatoid arthritis. Am J Pathol. 2013;183:857–67.

53. Hemingway F, Taylor R, Knowles HJ, Athanasou NA. RANKL-independent human osteoclast formation with APRIL, BAFF, NGF, IGF I and IGF II. Bone. 2011;48:938–46.

54. Fukushima Y, Quinn JM, Sabokbar A, McGee JO, Athanasou NA. The human osteoclast precursor circulates in the monocyte fraction. Endocrinology. 1996;137:4058–60.

55. Cai H, Xue Y, Wang P, Wang Z, Li Z, Hu Y, Li Z, Shang X, Liu Y. The long noncoding RNA TUG1 regulates blood-tumor barrier permeability by targeting miR-144. Oncotarget. 2015;6:19759–79.

56. Cesana M, Cacciarelli D, Legnini I, Santini T, Sthandier O, Chiapinna M, Tramontano A, Bozzoni I. A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. Cell. 2011;147:358–69.

57. Liang W, Fu W, Wang Y, Sun Y, Xu L, Gong C, Chan K, Li G, Weye MM, Zhang J. H19 activates Wnt signaling and promotes osteoblast differ- entiation by functioning as a competing endogenous RNA. Sci Rep. 2016;6:20121.

58. Kapinas K, Kessler C, Ricks T, Gronowicz G, Delany AM. miR-29 modulates Wnt signaling in human osteoblasts through a positive feedback loop. J Biol Chem. 2010;285:25221–31.

59. Asagiri M, Takayasu H. The molecular understanding of osteoclast dif- ferentiation. Bone. 2007;40:251–64.

60. Marie PJ. Osteoblast dysfunctions in bone diseases: from cellular and molecular mechanisms to therapeutic strategies. Cell Mol Life Sci. 2015;72:1347–61.

61. Walsh MC, Kim N, Kadono Y, Rho J, Lee SY, Lorenzo J, Choi Y. OSTEIMMU- NOLOGY: interplay between the immune system and bone metabolism. Annu Rev Immunol. 2006;24:53–63.

62. Hunter DJ, Felsen DT. Osteoarthritis. BMJ-Brit Med J. 2006;332:639–42.

63. Xing D, Liang J, Li Y, Lu J, Li H, Xu L, Ma X. Identification of long non- coding RNA associated with osteoarthritis in humans. Orthop Surg. 2016;4:288–93.
71. Meulenbelt IM, Bhutani N, den Hollander W, Gay S, Oppermann U, Reynard LN, Skelton AJ, Young DA, Beier F, Loughlin J. The first international workshop on the epigenetics of osteoarthritis. Connect Tissue Res. 2016;58:37–48.

72. Steck E, Bœuf S, Gabler J, Werth N, Schnatzer P, Diederichs S, Richter W. Regulation of H19 and its encoded microRNA-675 in osteoarthritis and under anabolic and catabolic in vitro conditions. J Mol Med. 2012;90:1185–95.

73. Katarzyna AD, Jérôme EL, Aïda MS, Christopher LM. Type II collagen expression is regulated by tissue-specific miR-675 in human articular chondrocytes. J Biol Chem. 2010;285:24381.

74. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet. 2005;366:1809–20.

75. Zou Y, Li C, Shu F, Tian Z, Xu W, Xu H, Tian H, Shi R, Mao X. IncRNA expression signatures in periodontitis revealed by microarray: the potential role of IncRNAs in periodontitis pathogenesis. J Cell Biochem. 2015;116:640–7.

76. Wang L, Wu F, Song Y, Li X, Wu Q, Duan Y, Jin Z. Long noncoding RNA related to periodontitis interacts with miR-182 to upregulate osteogenic differentiation in periodontal mesenchymal stem cells of periodontitis patients. Cell Death Dis. 2016;7:e2327.

77. Schaefer AS, Richter GM, Groesner-Schreiber B, Noack B, Nothnagel M, et al. Identification of a shared genetic susceptibility locus for coronary heart disease and periodontitis. PLoS Genet. 2009;5(2):e1000378.

78. Bochenek G, Hässler R, El Mokhtari N, König IR, Loos BG, Jepsen S, Rosenstiel P, Schreiber S, Schaefer AS. The large non-coding RNA ANRIL, which is associated with atherosclerosis, periodontitis and several forms of cancer, regulates ADIPOR1, VAMP3 and C11ORF10. Hum Mol Genet. 2013;22:4516–27.

79. Gibb EA, Brown CJ, Lam WL. The functional role of long non-coding RNA in human carcinomas. Mol Cancer. 2011;10:38–38.

80. Huanre M, Rinn JL. Large non-coding RNAs: missing links in cancer? Hum Mol Genet. 2010;19:R152–61.

81. Pasic I, Shlien A, Durbin AD, Stavropoulos DJ, Baskin B, Ray PN, Novokmet A, Malkin D. 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 Res. 2010;70:160–71.

82. 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. Tumor Biol. 2016;37:9441.

83. 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. Tumor Biol. 2015;36:1477.

84. Cai X, Liu Y, Yang W, Xia Y, Yang C, Yang S, Liu X. Long noncoding RNA MALAT1 as a potential therapeutic target in osteosarcoma. J Orthop Res. 2016;34:932–41.

85. Fang D, Yang H, Lin J, Teng Y, Jiang Y, Chen J, Li Y. 17β-estradiol regulates cell proliferation, colony formation, migration, invasion and promotes apoptosis by upregulating miR-9 and thus degrades MALAT-1 in osteosarcoma cell MG-63 in an estrogen receptor-independent manner. Biochem Biophys Res Commun. 2013;457:500–6.

86. Huyh NPT, Anderson BA, Guillak F, McAlinden A. Emerging roles for long noncoding RNAs in skeletal biology and disease. Connect Tissue Res. 2017;58:116–41.

87. Li Z, Yu X, Shen J. Long non-coding RNAs: emerging players in osteosarcoma. Tumor Biol. 2016;37:2811.

88. Mendenhall WM, Werning JW, Fernandes R, Malaya RS, Mendenhall NP. Ameloblastoma. Am J Clin Oncol. 2007;30:645–8.

89. Davanian H, Balasiddiaha A, Heymann R, Sundstrom M, Redenstrom P, Silfverberg M, Brolin D, Sallberg M, Lindskog S, Kruger Weiner C, et al. Ameloblastoma RNA profiling uncovers a distinct non-coding RNA signature. Oncotarget. 2017;8:4530–42.