The Long Non-coding RNA Inc-DMP1 Regulates Dmp1 Expression Through H3K27Ac Modification

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Several long non-coding RNAs (lncRNAs) have been reported regulate the expression of neighbor protein-coding genes at post-transcriptional, transcriptional and epigenetic levels. Dmp1 (Dentin matrix protein 1), encoding a non-collagenous extracellular matrix protein, plays an important role in dentin and bone mineralization. However, the transcriptional regulation of lncRNA on Dmp1 has not been reported. In this study, we identified a novel lncRNA named Inc-DMP1, which is near the Dmp1 gene region and undergoes remarkable changes during mandible development. Inc-DMP1 is co-localized and significantly expressed correlation with Dmp1 in embryonic and postnatal mouse mandibles. In MC3T3-E1 cells, Inc-DMP1 positively regulates DMP1 expression and skeletal mineralization. Furthermore, Inc-DMP1 induces the promoter activity of Dmp1 by modulating H3K27Ac enrichment in the Dmp1 promoter. In conclusion, our results indicate that Inc-DMP1 is a novel lncRNA near the Dmp1 gene region and regulates Dmp1 expression by modulating the H3K27 acetylation level of Dmp1 promoter.

Keywords: long non-coding RNA, Dentin matrix protein 1, transcriptional regulation, mice mandible, MC3T3-E1 cell, H3K27Ac

INTRODUCTION

Long non-coding RNAs (lncRNAs) are defined as a subgroup of non-coding RNA molecules that consist of at least 200 nucleotides and exhibit no or limited protein-coding capability (Roberts et al., 2014; Marchese et al., 2017). Since the major role of Xist in X-chromosome inactivation was first described (Brockdorff et al., 1992; Brown et al., 1992), studies have demonstrated that lncRNAs function in multiple cellular processes, such as genomic locus imprinting (Kanduri, 2016), antiviral response (Fortes and Morris, 2016) and differentiation and development (Fatica and Bozzoni, 2014). Studies have also investigated various mechanisms underlying lncRNA functions. Some nuclear lncRNAs are expressed from imprinted loci function as molecular scaffolds that recruit chromatin-modifying complexes and regulate gene expression in cis by altering the chromatin structures of target genes (Lee and Bartolomei, 2013; Melo et al., 2013). Other lncRNAs modulate gene expression in trans by interfering with transcriptional machineries or maintaining the structures of nuclear speckles (Prasanth et al., 2005; Clemson et al., 2009; Sunwoo et al., 2009). Some cytosolic lncRNAs have been suggested to regulate mRNA splicing, mRNA decay, protein translation and protein stability (Yoon et al., 2013; Quinn and Chang, 2016).

Dentin matrix protein 1 (DMP1), a highly phosphorylated acidic non-collagenous phosphoprotein, is initially identified by cDNA cloning in rat odontoblasts and highly expressed...
in bone tissues (George et al., 1993; D'Souza et al., 1997; Hirst et al., 1997; MacDougall et al., 1997; Feng et al., 1998). DMP1 plays a key role in the control of mineralization and phosphate homeostasis in dentin and bone (Qin et al., 2007). The deletion of Dmp1 leads to severe defects in the odontogenesis of the dentin and cartilage formation of bones in mice (Ye et al., 2004, 2005). Importantly, the loss of Dmp1 results in autosomal recessive hypophosphatemic rickets, a novel disorder in humans (Feng et al., 2006; Lorenz-Depiereux et al., 2006).

Narayanan et al. (2003) investigated the transcriptional regulation of Dmp1 by c-Fos and c-Jun, two AP-1 transcriptional factor family members, which play important roles in early osteoblast differentiation. They also demonstrated that JunB can interact with p300 and dramatically modulate the promoter activity of Dmp1 during osteoblast mineralization (Narayanan et al., 2002). The transcription factor TCF11 binds to the Dmp1 promoter and activates Dmp1 transcription in osteoblasts (Jacob et al., 2014). However, the effect of IncRNA on the transcriptional regulation of Dmp1 has yet to be reported.

Since the transcription factor JunB regulates the expression of Dmp1 gene through interaction with the transcriptional coactivator p300, which is a histone acetyltransferase (Narayanan et al., 2002). Meanwhile, IncRNAs could function as molecular scaffolds of histone-modifying enzyme to modulate the expression of target gene through specific histone modification such as methylation and acetylation (Marchese et al., 2017). So, we assumed that IncRNAs could play a certain regulatory role in the expression of Dmp1. Thus, we used IncRNA-seq technology to screen IncRNAs near the promoter of Dmp1 and differently expressed between embryonic and postnatal mouse mandibles. Inc-DMP1 was selected on the basis of the IncRNA sequence profiles. Then, the expression patterns of Inc-DMP1 and Dmp1 in the embryonic and postnatal mouse mandibles were detected through qRT-PCR and RNA-fluorescence in situ hybridization (FISH) to investigate whether Inc-DMP1 was significantly correlated with Dmp1. Subsequently, whether Inc-DMP1 could participate both mRNA and protein expression of DMP1 and mineralization were tested in osteoblast cells MC3T3-E1. Finally, luciferase assay and chromatin immunoprecipitation (ChIP) methods were performed to investigate whether Inc-DMP1 could transcriptionally regulate the Dmp1 expression in MC3T3-E1 cells by modulating the enrichment of H3K27Ac in Dmp1 promoter.

**MATERIALS AND METHODS**

**Animals and Cell Lines**

Embryonic (16, 18, and 20 days old) and postnatal (2 and 4 weeks old) C57 mice were purchased from the Animal Experiment Center of Sun Yat-sen University (Guangzhou, China). The body weight of the mice was 10–15 g, and the mice were fed at room temperature (20–22°C) (License code: SYXX: 2012-0081). The mice were anesthetized by intraperitoneally injecting 5% chloral hydrate (0.1 mL/10 g) and then fixed on a dry ice anatomy bench. The mandibles were extracted under a microscope, frozen in liquid nitrogen and stored at −80°C. After sampling was completed, the mice were sacrificed by dislocating their necks and then treated in accordance with the death treatment method for experimental animals. All animal experiments were conducted following the Ministry of Health national guidelines for housing and care of laboratory animals and performed in accordance with institutional regulations after review and approval by the Institutional Animal Care and Use Committee at the Sun Yat-sen University (IACUC-DB-13-132).

The MC3T3-E1 cell line (American Type Culture Collection, Manassas, VA, United States) was cultured in α-MEM (Gibco, New York, NY, United States) and pretreated with 10% fetal bovine serum. The cells were cultured in an incubator with humidified atmosphere and 5% CO₂ at 37°C.

**IncRNA Sequencing Assay**

Total RNA was isolated from the mandibles of embryonic (18 days old) and postnatal (2 weeks old) C57 mice with TRIzol reagent (Thermo Fisher Scientific, Carlsbad, CA, United States) in accordance with the protocol. RNA quantity and quality were evaluated with Agilent Bioanalyzer 2100. The qualified RNA samples were purified and synthesized to cDNA. After cluster amplification was conducted through PCR, a sequencing library was sequenced with Illumina HiSeqTM2000. Raw sequences were filtered to remove the joint, undetected sequences and low-quality sequences. Transcriptional transcripts were constructed from the remaining sequence, and known non-coding RNAs and protein-encoding fragments were discarded. The new non-coded sequence and the known non-coding sequence were compared and statistically analyzed to screen the differentially expressed IncRNAs in the two-time-point tissue samples.

On the basis of the Poisson distribution model, we simultaneously calculated the differential expression multiples [log2(E18D/P2W)] of IncRNA in E18D and P2W samples in accordance with the gene expression (RPKM value) and examined the differences in P-value by controlling the false discovery rate (FDR) to determine the P-value of the domain. IncRNAs with | log2(E18D/P2W) | >4 and FDR < 0.01 were considered differentially expressed in E18D and P2W samples.

**RNA-FISH Assay**

The mandible samples of embryonic (16, 18, and 20 days old) and postnatal (2 and 4 weeks old) C57 mice were collected and fixed in 4% paraformaldehyde for 48 h and then decalcified in EDTA. The decalcified specimens were paraffin embedded, and 5 mm serial sections were prepared for the experiments. The slides were hybridized with Inc-DMP1 and Dmp1 probes overnight at 37°C. Afterward, the slides were washed twice with 2 × saline sodium citrate (SSC) and three times with deionized water formamide: 2 × SSC (50:50) for 20 min. DAPI solution was used to stain the cell nuclei. Fluorescence images were acquired through fluorescence microscopy.

**Overexpressing and RNAi of Inc-DMP1**

For Inc-DMP1 overexpression, the full length of Inc-DMP1 was cloned into the lentivirus vector pCDH-CMV-MCS-EF1-copGFP...
(Gene, Shanghai, China). The shRNA sequence targeting lnc-DMP1 was cloned into the lentivirus vector psi-LVR6MH (Gene) for the interference of the lnc-DMP1 expression. All of the vectors were transfected into MC3T3-E1 cells in accordance with the manufacturer's instructions. The efficiency of transfection was confirmed through qRT-PCR. The cells were subjected to RNA extraction, Western blot assay and functional assays.

Quantitative Real-Time PCR Assay (qRT-PCR)

The total RNA of mandible samples and cells was extracted with TRIzol reagent in accordance with the manufacturer's instructions (Thermo Fisher Scientific) and reverse transcribed with a high-capacity cDNA reverse transcription kit (Vazyme, Nanjing, China). SYBR Green PCR master Mix (Vazyme) and primers (Table 1) were used for qRT-PCR. The qRT-PCR program was set at the following parameters: 95°C for 2 min, followed by 40 cycles at 95°C for 15 s and 60°C for 32 s. GAPDH was used as reference genes, and the relative gene expression level was calculated by using the comparative threshold (CT) cycle method (2−ΔΔCT).

Western Blot Assay

The cultured cells were extracted by RIPA (Radio Immunoprecipitation Assay) ordinary type reagent (Vazyme), which is a traditional cell tissue lysis buffer to gain proteins for western and IP experiments. Then, 30 µg of each protein was loaded to each lane, fractionated by 10% SDS-PAGE, transferred onto PVDF membranes and probed with the primary antibody of DMP1 and DSPP (Abcam, Cambridge, United Kingdom).

Luciferase Assay

The dual-luciferase reporter vector pGL3-Basic vector, which contains renilla luciferase with 35S promoter and firefly luciferase without driven promoter, was used to construct the pGL3-Dmp1 reporter vector that including both renilla luciferase with 35S promoter and firefly luciferase driven by the Dmp1 promoter (2000 bp before transcription start site). The plasmids of CMV empty vector, CMV-lnc-DMP1, U6-Sh empty vector and U6-Sh-lnc-DMP1 were co-transfected with pGL3-Dmp1 plasmid into MC3T3-E1 cell. Lipofectamine 2000 was used. After 48 h of transfection, the cells were collected, and luciferase activity was measured by using a dual luciferase reporter assay kit (Promega, Madison, United States) in accordance with the manufacturer’s protocol. The firefly luciferase activities of the Dmp1 promoter co-transfected with CMV vectors or U6-Sh vectors were firstly normalized to renilla luciferase activity of itself, then showed as a relative LUC activity to the empty vector control CMV-lnc-DMP1 or U6-Sh empty vector.

DNA Pull Down

In vitro, part of purified PCR product Dmp1 DNAs, which was 378 bp length of Dmp1 promoter before the transcriptional start site (TSS), were biotin-labeled with a Biotin DNA labeling mix (Roche, Basel, Switzerland). The Biotin-labeled and unbiotin-labeled Dmp1 DNAs were added for 2 h. The blots were visualized through enhanced chemiluminescence and autoradiography.

Induction of Odontoblast-Like Differentiation and Alizarin Red Staining

The lnc-DMP1 overexpression and RNAi stable cell lines were induced by osteoinductive factors (50 µg/mL ascorbic acid phosphate, 10 mM β-glycerol phosphate disodium salt and 10 mmol/L dexamethasone) for 28 days. After mineralization was induced, the medium was discarded. The cells were rinsed with PBS three times, fixed with 95% ethanol for 20 min and stained in alizarin red solution for 10 min. The calcification nodules formed in each group were compared.

ChIP

ChIP was conducted by using a ChIP kit (Qiagen, Düsseldorf, Germany) in accordance with the manufacturer’s instructions. In brief, the cells were incubated with formaldehyde, and the link between DNA and protein was built. Then, the cross-linked chromatin was sonicated into fragments. Anti-H3K27ac antibodies (Abcam) were used for the immunoprecipitation of the chromatin fragments, and IgG was used as the negative control. Subsequently, the precipitated chromatin DNA was measured through qRT-PCR.

TABLE 1 | Sequences of primers used in this study.

| Primer name | Sequence (5'-3') | Purpose |
|-------------|-----------------|---------|
| Dmp1-Q-F    | GCGTGTGTTACGCCGGCCTGGGAATGAC | qRT-PCR |
| Dmp1-Q-R    | CCGCTGCTGGCTAGTACCGAAA | qRT-PCR |
| Inc-DMP1-Q-F | TTCGCCACAGCTAGACGACAA | qRT-PCR |
| Inc-DMP1-Q-R | CTTAAGCTGCTAGCAGGAGA | qRT-PCR |
| GADPH-Q-F   | GGCCCTCCTCTTATGTTTGG | qRT-PCR |
| GADPH-Q-R   | GGCCTCCCTCTTATGTTTGG | qRT-PCR |
| pCDH-Inc-DMP1-F | AAAAAACGAGAACGGGAAAAC | Inc-DMP1 OE |
| pCDH-Inc-DMP1-R | GAGCCTCGTTCTACTATG | Inc-DMP1 OE |
| sh-Inc-DMP1-F | AAAAAACGAGAACGGGAAAAC | Inc-DMP1 RNAi |
| sh-Inc-DMP1-R | GAGCCTCGTTCTACTATG | Inc-DMP1 RNAi |
| PGL3-pro-Dmp1-F | GGCAATATGCTCTACATTTC | Luciferase assay |
| PGL3-pro-Dmp1-R | GGCAATATGCTCTACATTTC | Luciferase assay |
| Dmp1-p-F    | CGGAAATCATTTATTTTGAAGG | ChiP-qRT-PCR |
| Dmp1-p-R    | CGGAAATCATTTATTTTGAAGG | ChiP-qRT-PCR |
| GAPDH-p-F   | CTGCCCTGACAGGCGCTGACAG | ChiP-qRT-PCR |
| GAPDH-p-R   | CTGCCCTGACAGGCGCTGACAG | ChiP-qRT-PCR |
(Thermo Fisher Scientific) was added to each binding reaction and further incubated at RT for 1 h. The beads were washed briefly with wash buffer [RIP buffer (100 mM NaCl, 10 mM Tris, 1 mM EDTA, 1 mM EGTA, pH 7.6) with 0.1% TritonX 100] five times and boiled in SDS buffer. The retrieved proteins were detected by Western blot.

**Primer Sequences**
The primer sequences are shown in Table 1.

**Statistical Analysis**
Data were analyzed with SPSS 20.0 and presented as mean ± SD. Differences between the two groups were assessed by using two-tailed Student’s t-test. Differences with a P < 0.05 were considered statistically significant (*P < 0.05 and **P < 0.01).

**RESULTS**

**Expression Profile Analysis of IncRNAs in the Development of Mouse Mandibles**
To examine the expression profiles of IncRNAs in the development of mouse mandibles, we performed IncRNA sequencing (IncRNA-seq) analysis. RNA was collected from the mouse mandibles of embryonic 18-day-old (E18D) and postnatal 2-week-old (P2W) mice. A total of 38,566 IncRNAs were identified in the two samples (Figure 1A). Through stringent classification (| log2 fold change (E18D/P2W) | > 4, FDR < 0.001), 808 and 408 IncRNAs were found to be up-regulated (log2 fold change (E18D/P2W) > 4) and down-regulated (log2 fold change (E18D/P2W) < -4) (Figure 1B), respectively. These results suggested that IncRNAs might be involved in the regulation of mouse mandible development.

**Inc-DMP1 Differentially Expressed in the Development of Mouse Mandibles and Located Near the Dmp1 Gene Region**
To investigate IncRNAs that might modulate the transcriptional expression of Dmp1 in cis, we searched IncRNAs located within 100 kb of the Dmp1 gene region (Chromosome 5:104202613-104214102) from the IncRNA-seq data. We found that Inc19585 and Inc741 were located downstream of Dmp1, with distances of 16.7 and 45.6 kb, respectively, whereas Inc30475 was located upstream of Dmp1, with distance of 68.6 kb (Figure 1C). Amongst these three IncRNAs, Inc19585 was the nearest to Dmp1 and the only one that was up-regulated in the E18D sample relative to the P2W sample. Thus, we renamed Inc19585 as Inc-DMP1.

To confirm the results of the Inc-DMP1 expression from IncRNA-seq, we also conducted qRT-qPCR to detect the expression levels of Inc-DMP1 and Dmp1 in the mandibles derived from E18D and P2W mice. The results showed that the level of Inc-DMP1 was remarkably up-regulated in E18D compared with that in P2W (Figure 1D), and this expression pattern was consistent with that of Dmp1 (Figure 1E). These results indicated that Inc-DMP1 was a novel IncRNA near the Dmp1 gene region, which was differentially expressed in the development of mouse mandibles.

**Co-localization of Inc-DMP1 and Dmp1 in the Development of Mouse Mandibles**
To test whether Inc-DMP1 was co-localized with Dmp1, we performed RNA-FISH to detect the expression of Inc-DMP1 and Dmp1 in the mandibles derived from embryonic 16-, 18-, and 20-day-old mice and postnatal 2- and 4-week-old mice. In Figure 2, the expression levels of Dmp1 (green fluorescence) and Inc-Dmp1 (red fluorescence) were highly co-localized (yellow fluorescence) in all of the developmental processes of mouse mandibles, and the co-localization was distributed in the region, including the nuclei (blue fluorescence) and the cytoplasm. We found that the expression levels of Inc-DMP1 and Dmp1 decreased in the postnatal mouse mandibles (Figures 2D,E) compared with those in the embryonic mouse mandibles (Figures 2A–C). These data suggested that the expression of Inc-DMP1 was significantly correlated with that of Dmp1 in different developmental processes of mouse mandibles.

**Inc-DMP1 Positively Regulates Dmp1 Expression and Mineralization in MC3T3-E1 Cells**
To test the role of Inc-DMP1 on the modulation of Dmp1 expression, we selected MC3T3-E1 cells to construct stable OE and RNAi lines of Inc-DMP1, which were, respectively, transduced with the expression vectors of pCDH-CMV-Inc-DMP1 and psi-LVRU6MH-sh-Inc-DMP1, and the empty vectors were transduced as control lines (Figure 3A). The mRNA expression level of Dmp1 was detected in stable Inc-DMP1 OE and RNAi lines, and qRT-PCR results showed that the Dmp1 expression increased in the Inc-DMP1 OE line and decreased in the Inc-DMP1 RNAi line relative to the control lines (Figure 3B). Dspp was a downstream gene of Dmp1, whose expression was regulated by Dmp1. We also detected the mRNA expression level of Dspp in Inc-DMP1 OE and RNAi lines, and the results showed the same expression pattern of Dspp as Dmp1 in these stable lines (Figure 3C). We further tested the alteration on the protein level through an immunoblot assay. The results showed that the protein levels of DMP1 and DSPP increased in the Inc-DMP1 OE line and decreased in the Inc-DMP1 RNAi line (Figure 3D). These data suggested that Inc-DMP1 acted as an upstream regulator promoted both mRNA and protein expression of DMP1.

Given that Dmp1 plays an important role in the control of osteoblast cell mineralization (Toyosawa et al., 2001; Ling et al., 2005), we determined whether Inc-DMP1 participated in the skeletal mineralization of osteoblast cells. The results of the cell mineralization assays performed after the cells were treated with osteo-inductive factors for 28 days showed that more mineralized nodules were formed in the Inc-DMP1 OE line compared with those in the control lines, whereas the Inc-DMP1 RNAi line showed obviously inhibited mineralized nodules formation compared with that in the control lines (Figure 3E). These results suggested that Inc-DMP1 controlled
mineralization by increasing the \textit{Dmp1} expression in MC3T3-E1 cells.

### Roles of Inc-DMP1 in Modulating the Activity and H3K27ac Enrichment of \textit{Dmp1} Promoter

To test whether Inc-DMP1 functions in the transcriptional regulation of \textit{Dmp1} expression, we made a construct expressing a luciferase reporter gene under the control of the \textit{Dmp1} promoter. While the \textit{pDmp1:LUC} reporter gene was co-transfected with CMV-\textit{Inc-DMP1} plasmid into MC3T3-E1 cells, the expression of luciferase significantly increased relative to that of the control. The co-transfected of U6-sh-\textit{Inc-DMP1} plasmid reduced the reporter gene expression (Figure 4A). These results suggested that Inc-DMP1 induced the promoter activity of \textit{Dmp1}.

\textit{IncRNA} can bind adaptor protein and target transferase to drive histone modification and active gene transcription
FIGURE 2 | RNA-FISH assay of Inc-DMP1 and Dmp1 in the development of mouse mandibles. Representative images of Inc-DMP1 (green) and Dmp1 (red) expression in embryonic 16- (A), 18- (B) and 20-day (C) mouse mandibles and in postnatal 2- (D) and 4-week (E) mouse mandibles. Yellow denoted the co-localization of Inc-DMP1 and Dmp1. Nuclei were stained with DAPI (blue). Scale bar represented 500 µm.

(Marchese et al., 2017). According to the ENCODE (Encyclopedia of DNA Elements) project data, we could not find any histone modification information of Dmp1 in mouse cells. Nonetheless, histone H3-lysine-27 acetylation (H3K27Ac) was enriched in the promoter region of Dmp1 in human H1-hESC cells. Hence, we conducted a ChIP assay to detect the enrichment of H3K27Ac in the promoter of Dmp1 in MC3T3-E1 cells. The results showed that the acetylation of H3K27 in the promoter of Dmp1
FIGURE 3 | Inc-DMP1 controls mineralization by increasing the DMP1 expression in MC3T3-E1 cells. (A) The transformation efficiency of Inc-DMP1 OE and RNAi stable lines in MC3T3-E1 cells was determined through qRT-PCR. Data were normalised to GAPDH expression, and the empty vector control was set to a value of 1. (B,C) The alteration of the mRNA levels of Dmp1 (B) and Dspp (C) in Inc-DMP1 OE and RNAi cells. (D) The alteration of the protein levels of DMP1 and DSPP in Inc-DMP1 OE and RNAi cells. (E) Mineralization was detected by subjecting Inc-DMP1 OE and RNAi cells to alizarin red staining. **P < 0.01 by Student's t-test.

was enriched (Figures 4B,C). The DNA pull down assay also confirmed the binding of Dmp1 promoter to the H3K27Ac protein (Figure 4D).

Given that the enhanced H3K27Ac histone modification is usually an important agonist for transcriptional regulation, we hypothesized that Inc-DMP1 participated in the acetylation of H3K27 in the promoter of Dmp1. To confirm this hypothesis, we performed ChIP assay to detect the enrichment of H3K27Ac in the promoter of Dmp1 in Inc-DMP1 OE and Inc-DMP1 RNAi stable lines. As shown in Figure 4E, the overexpression of Inc-DMP1 increased the enrichment of H3K27Ac of the Dmp1 promoter, whereas the RNAi of Inc-DMP1 decreased the enrichment of H3K27Ac of the Dmp1 promoter. These results suggested that Inc-DMP1 induced the promoter activity by modulating the H3K27Ac enrichment of Dmp1 promoter.

DISCUSSION

IncRNAs play important regulatory roles during multiple biological processes and in various diseases (Wang et al., 2015; Ding et al., 2017). Histone modification through methylation and acetylation is the main regulatory mechanism by which IncRNAs regulate the expression of their neighbor protein-coding genes. IncRNAs modulate the expression of target genes by promoting the acetylation of H3K27 in the promoter regions of genes. Dmp1 is highly expressed in bone and dentin tissues and plays a key role in mineralization, but the mechanism of its regulation needs to further investigated (Narayanan et al., 2002; Jacob et al., 2014; Wang et al., 2014). In this study, we attempted to investigate the potential regulatory pattern in IncRNAs and Dmp1.

Inc-DMP1, a novel IncRNA validated by the IncRNA sequence in our study, was near the Dmp1 gene region and exhibited the most remarkable changes during mandible development. The RNA-FISH assay demonstrated that Inc-DMP1 and Dmp1 were expressed in the same areas in the mandibles and co-localized in the cytoplasm and the nuclei. Moreover, the significant correlation between the expression levels of Inc-DMP1 and Dmp1 at different stages of mandible development implied the presence of a regulatory mechanism between them. After constructing the stable lines of Inc-DMP1 overexpression and RNAi in osteoblast cell lineMC3T3-E1, we found that overexpressing Inc-DMP1 promoted both mRNA and protein expression of DMP1 and skeletal mineralization in MC3T3-E1.
cells. These results indicated that lnc-DMP1 positively regulated the expression of DMP1 to control the mineralization. The luciferase results implied that lnc-DMP1 induced the promoter activity of Dmp1. Given that the acetylation level of H3K27 was positively correlated with the transcriptional activity, the results of the ChIP assays confirmed our hypothesis that lnc-DMP1 enhanced Dmp1 expression by enriching H3K27Ac in Dmp1 promoter.

In summary, our study identified a novel lnc-DMP1 positively related to the Dmp1 during the development of mouse mandibles. The lnc-DMP1 regulated Dmp1 expression and mineralization by modulating the H3K27ac enrichment of Dmp1 promoter in osteoblast cells. In our future studies, we will further investigate chromatin-modifying complexes, which interact with lnc-DMP1 to modulate Dmp1 expression. Collectively, our findings will serve as a basis for exploring the mechanism of lncRNAs in...
the regulation of bone regeneration, including periodontal tissue reconstruction, and for tissue engineering and clinical practice.

DATA AVAILABILITY STATEMENT

The Inc-RNA sequencing data mentioned in our manuscript has been submitted to the SRA in NCBI, the SubmissionID is BioProject ID is PRJNA540617 and BioSample accessions are SAMN11549457 and SAMN11549458.

AUTHOR CONTRIBUTIONS

XX, YR, BL, YY, XK, and PZ performed the experiments and/or analyzed the data. HW designed the research. YR participated in the design of some experiments. XX and HW wrote the manuscript. All co-authors corrected the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.