Bone remodeling regulated by long non-coding RNAs

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Abstract: Long non-coding RNAs (lncRNAs) are a new class of non-coding RNA molecules in metazoan genome and might play widespread roles in gene regulation and other cellular processes. This review will highlight our current understanding of lncRNA biology, mechanisms of action, and summarize recent work on the role of lncRNAs including lncRNA ANCR in bone remodeling. These studies epitomize emerging importance of lncRNAs in the regulation of osteoblast differentiation and function, indicating new potential targets for bone wound healing.
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1. Introduction

Normal healing of bone is characterized by the integrated actions of different cells, and its processes are divided into broad sequential phases of inflammation, proliferation, and migration of osteogenic cells, and production and remodeling of trabecular bones (Florencio-Silva et al., 2015; Raisz, 1999). Bone remodeling, or turnover, is mediated by osteoblast and osteoclast numbers and activities to reach dynamic balance (Figure 1).

osteoblasts continue to differentiate, the matrix

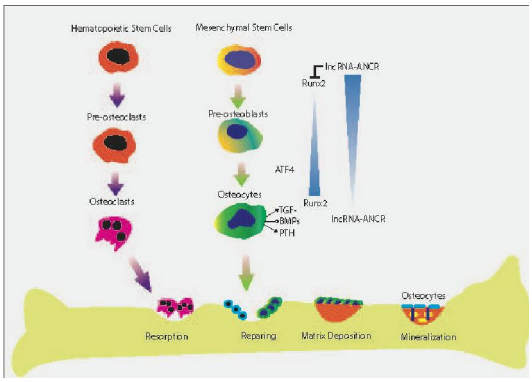

Figure 1. **The bone remodeling process of resorption by osteoclasts and formation by osteoblasts.** Osteoblasts are derived from mesenchymal stem cells regulated by transcription factor Runx2 and non-coding RNAs such as long non-coding RNAs (e.g., lncRNA-ANCR).

Like any engineering process, deconstruction is followed by construction to create a new object. In bone healing process, two main workers: Osteoclasts resorb bone, whereas osteoblasts make new bone. Osteoclasts are recruited by cytokines at the site of remodeling to the bone surface, then the osteoclasts form a disarranged boarder that allows their tight adherence to the bone surface (Capulli et al., 2014). An acidic environment generated by the osteoclast dissolves the mineralized component of the bone matrix in the damaged bone area. The organic matrix becomes exposed and is subsequently degraded. The next phase of bone remodeling is the reversal phase, it begins with mononuclear cells that prepare the bone surface for new osteoblasts and provide signals to recruit them. Once a pool of osteoblast progenitors expressing Runx2 and CollA1 has been established during osteoblast differentiation, there is a proliferation phase. During this phase, preosteoblasts are developed with alkaline phosphatase activity, then transit to mature osteoblasts which is characterized by an increase in the expression of Osx and in the sectetion of bone matrix proteins such as osteocalcin, bone sialoprotein I/II and collagen type 1.

As the matures and is mineralized. Once the bone surface is restored, mature osteoblasts can undergo apoptosis or terminally differentiate into either bone surface lining cells or osteocytes, which are embedded in the calcified matrix and are responsive to mechanical stresses (Buckwalter et al., 1996).

Osteoclasts are derived from the monocyte/Macrophage of the hematopoietic stem cell lineage. Osteoclasts differentiation is under the influence of several factors and dependent on multiple extracellular signaling molecules, including macrophage colony-stimulating factor (MCSF) secreted by osteoprogenitor mesenchymal cells, receptor activator for nuclear factor κB ligand (RANKL), tumor necrosis factor, interferon gamma, and interleukins secreted by osteoblasts, osteocytes, and stromal cells. Together, these factors promote the

activation of transcription factors and gene expression in osteoclasts (Florencio-Silva et al., 2015).

Osteoblasts are derived from mesenchymal stem cells (MSCs), which can differentiate into osteoblasts, adipocytes, chondrocytes, or myocytes, depending on the activation or inhibition of specific signaling pathways. Some of the most important signaling molecules regulating osteoblastic cell differentiation include bone morphogenetic proteins (BMPs), transforming growth factor (TGF)-β, members of the Wingless (Wnt) pathways, Hedgehog, parathyroid hormone, insulin-like growth factor-1, fibroblast growth factors, and Notch. An imbalance of bone resorption and formation results in several bone diseases. For example, excessive resorption by osteoclasts contributes to bone loss and osteoporosis (Carnesecchi and Vanacker, 2015), whereas the excessive bone formation by osteoblasts may results in osteopetrosis (Sobacchi et al., 2013). Emerging evidence demonstrated that the bone wound healing process, or bone remodeling processes are regulated by non-coding RNAs including microRNAs and long non-coding RNAs. microRNA regulation in osteogenesis has been well described in recently review paper (Kapinas and Delany, 2011) and beyond the scope of this article. Here we focus on lncRNA biology and summarize the latest findings of lncRNA regulation in osteoblast differentiation.

2. Long non-coding RNAs in the genome

Approximately two-thirds of genomic DNA is pervasively transcribed into various RNA molecules in human cells (Djebali et al., 2012). LncRNAs are the most recently appreciated new class of non-coding RNAs, yet the most diverse molecules. The latest annotation of the human genome (Version 21, GRCh38—Ensemble 77, http://www.gencodegenes.org/stats.html) identified 26,414 transcripts from 15,877 lncRNA genes. LncRNA genes are typically shorter than protein-coding genes and have fewer exons, typically only 2-3 (Cabili et al., 2011; Derrien et al., 2012). LncRNA transcripts may be transcribed from intergenic, intronic or antisense of protein coding genes. LncRNA genes are transcribed by RNA polymerase II, the primary RNA molecules are processed, spliced and polyadenylated. The length of lncRNA transcripts are between 200 bp to 100kb with complex secondary structures (Derrien et al., 2012). LncRNA expression is more cell type specific than the expression of protein-coding genes and lncRNAs are typically coexpressed with their neighboring genes albeit to an extent similar to that of pairs of neighboring protein-coding genes (Cabili et al., 2011). LncRNAs are distributed either in nucleus or cytoplamas, they no longer are 'transcription noise', in contrast, lncRNAs may interact with proteins, transcription factors, other RNA or DNA molecules, acting as guidance, decoys, scaffolds (Ulitsky and Bartel, 2013). As illustrated in Figure 2, lncRNA HOTAIR (Rinn et al., 2007), Xist (Zhao et al., 2008), bind to polycomb repressor complexs (PRC2) and modulate chromatin architecture, leading to inhibition of target genes. lncRNA Gas5 binds glucocorticoid receptor and takes this transcript factor away from promoters of target genes, thus shut down downstream gene expression (Kino et al., 2010). lncRNA MALAT-1 (Ji et al., 2003) regulates alternative splicing by modulating serine/arginine splicing factor phosphorylation (Tripathi et al., 2010). A group of lncRNAs with Alu repeat-containing sequence bind to 3' UTRs of target mRNAs mediated by the Staufen 1 protein and activate mRNA decay pathway (Gong and Maquat, 2011). Some lncRNAs can enhance (e.g., BACE-1AS) or prevent (e.g., lncRNA-p21) translation process. Taking together, lncRNAs are increasingly recognized as key regulators of gene expression at all levels - transcriptional, post-transcriptional and post-translational (Rinn and Chang, 2012). They may be novel 'fine-tuners' of cell fate to control cell identity and lineage commitment (Fatica and Bozzoni, 2014).

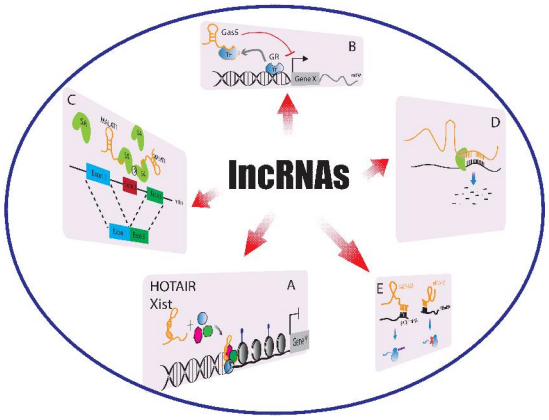

Figure 2. **The mechanisms of action of long coding RNAs.** LncRNAs may faction as decoys, scaffolds to interact with transcription factors, chromatin modifying factors or other RNAs, leading to gene expression regulation, RNA splicing, RNA stability, translation control. Therefore, lncRNAs have a variety of biological effects.

3. Long non-coding RNA and osteogenesis

LncRNAs have been shown to regulate cellular differentiation in various contents such as neuronal differentiation, muscle differentiation (Cesana et al., 2011). The involvement of lncRNAs during osteogenesis has become the interest of research. In early 2009, Babajko et al identified osteogenic regulator lncRNA, Msx1-AS, which negatively regulates its counterpart, Msx1 gene expression

(Babajko et al., 2009) and further abolish the inhibition of Dlx5 gene expression by Msx1. More recently, Zuo et al utilized high throughput lncRNA array to identify 116 lncRNAs differentially expression in C3H10T1/2 mouse mesenchymal stem cells undergoing BMP2-induced early osteoblast differentiation, among them, 24 lncRNAs exhibit co-expression with their nearby mRNAs in the same direction. For example, mouse lincRNA0231 and its nearby gene, EGFR, both were downregulated, while the expression of mouse lncRNA NR_027652 and its nearby gene DLK1 at chromosome 12 were increased simultaneously(Zuo et al., 2013). Interestingly, mouse lncRNA NR_027652, a genetic imprinted gene, is transcribed from maternally expressed 3(Meg3) gene, its human homologue MEG3 is in human chromosome 14q. In human, MEG3 and DLK1 locate at the same genomic locus. The reciprocal expression pattern controls cell differentiation and cell growth, therefore, they are acting as tumor suppressor (Zhou et al., 2012). Although the detailed functions of these lncRNAs during osteoblast cell differentiation need to be delineated, but the observation suggests lncRNAs may act as regulatory mechanisms in the control of osteoblast differentiation.

LncRNA ANCR, also known as anti-differentiation noncoding RNA, was well studied in osteoblast differentiation. ANCR is required to maintain the undifferentiated cell state within the epidermis (Kretz et al., 2012). Zhu et al found that ANCR expression was higher in human fetal osteoblastic cell line hFOB1.19 and its expression levels were significantly decreased in differentiated hFOB1.19 cells. Depletion of endogenous ANCR expression in hFOB1.19 cells resulted in spontaneous differentiation (without differentiation medium) with increase of osteoblast differentiation markers such as alkaline phosphatase and osteocalcin (Zhu and Xu, 2013). Overexpression of ANCR could prevent osteoblastic cell differentiation even under BMP-2 stimulation. The authors further investigated the underlying mechanisms of ANCR-mediated anti-differentiation and demonstrated that ANCR physically interacts with enhancer of zeste homolog 2 (EZH2), a histone-lysine methyltransferase enzyme, resulting in suppression of a key transcription factor Runx2 through its promoter region methylation. Therefore, ANCR acts as a scaffold to bring enzymatic protein EZH2 to promoter region of Runx2 and inhibits target gene expression, functions as a molecular switch regulating cell fate commitment. To date, 33 lncRNAs including HOTAIR have been associated with osteoblast differentiation (http://mlg.hit.edu.cn/lncrna2function/viewlncrna?go_id=GO:0001649), more experiments need to be done for functional studies in the future.

4. Discussions

Bone remodeling is a crucial process during bone wound healing, involving osteoclast cells and osteoblast cells formation. Osteoblast differentiation is regulated by many factors including bone morphgenetic protein-2, parathyroid hormone, and Runt-related transcription factor 2. It is evident from a relatively small studies suggested that lncRNAs have a significant and crucial role in the maintenance, commitment and differentiation of osteoblast cells into mature lineages. Understanding the mechanism of lncRNA action in osteoblastic cell differentiation is important to bone remodeling and bone wound healing. With more lncRNAs discovered in the process of bone remodeling, it is conceivable that manipulation of lncRNAs could be applied to therapeutic implication.

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