Bone xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Review

Chromatin modifiers and histone modifications in bone formation, regeneration, and therapeutic intervention for bone-related disease

Jonathan A.R. Gordon [a,*], Janet L. Stein [a], Jennifer J. Westendorf [b], Andre J. van Wijnen [b]

[a] Department of Biochemistry, University of Vermont, Burlington, VT, USA
[b] Department of Orthopedic Surgery, Mayo Clinic, Rochester, MN, USA

ARTICLE INFO

Article history:
Received 13 January 2015
Accepted 13 March 2015
Available online xxxx

Keywords:
Epigenetics
Chromatin
Chromatin-modifiers
Osteogenic lineage cells
Acetylation
Methylation

ABSTRACT

Post-translational modifications of chromatin such as DNA methylation and different types of histone acetylation, methylation and phosphorylation are well-appreciated epigenetic mechanisms that confer information to progeny cells during lineage commitment. These distinct epigenetic modifications have defined roles in bone, development, tissue regeneration, cell commitment and differentiation, as well as disease etiologies. In this review, we discuss the role of these chromatin modifications and the enzymes regulating these marks (methyltransferases, demethylases, acetyltransferases, and deacetylases) in progenitor cells, osteoblasts and bone-related cells. In addition, the clinical relevance of deregulated histone modifications and enzymes as well as current and potential therapeutic interventions targeting chromatin modifiers are addressed.

Contents

Introduction

The study of epigenetics has been widely defined as any heritable changes in an organism that occur without direct alteration in DNA sequence. This broad definition encompasses a wide range of chemical modifications of proteins and DNA that can alter the structure and accessibility of chromatin in the nucleus leading to altered gene expression. A great deal of research has defined multiple epigenetic mechanisms regulating transcription factor and transcriptional machinery access to chromatin, including nucleosome positioning [1,2], chromatin–chromatin interactions, lncRNA and miRNA binding [3,4], and several unique chemical post-translational modifications to DNA and DNA-associated proteins [5]. As these epigenetic mechanisms are too complicated to cover in brief, this review will focus on the two most prominent (and therefore widely studied) epigenetic modifications: DNA methylation and post-translational modifications of histone

* Corresponding author at: Department of Biochemistry, University of Vermont, Given Building, Room E210C, 89 Beaumont Drive, Burlington, VT 05405, USA.
E-mail addresses: Jonathan.A.Gordon@uvm.edu (J.A.R. Gordon), Janet.Stein@uvm.edu (J.L. Stein), Westendorf.Jennifer@mayo.edu (J.J. Westendorf), vanwijnen.andre@mayo.edu (A.J. van Wijnen).

http://dx.doi.org/10.1016/j.bone.2015.03.011
8756-3282/

proteins (i.e. lysine acetylation and methylation, and serine phosphorylation) as they relate to bone development, regeneration and disease.

DNA methylation

Methylation of cytosine bases at the fifth carbon (5-methylcytosine or 5mC) is a highly conserved DNA modification found in all mammals [6]. In somatic cells, cytosine methylation is essentially limited to palindromic CpG dinucleotides, however non-CpG methylation has been observed in some cells including: oocytes, pluripotent embryonic stem cells (ESCs), and mature neurons [7,8]. DNA methylation is dynamically regulated during development and contributes to developmental stage- and cell-type specific epigenetic signals that profoundly impact gene expression [9]. Nearly 70% of annotated gene promoter regions and a large number of transcriptional start sites (TSS) are associated with short CpG-dense sequences [10]. This would suggest that a large number of genes are susceptible to methylation at these CpG islands (CGIs), however CGIs are frequently non-methylated, regardless of the transcriptional state of the corresponding genes [10]. There are several examples of CGI methylation during normal development resulting in stable silencing of gene transcription, however it would appear that CGI methylation may not be an initiating event in gene silencing, but rather acting to permanently maintain gene inactivation [10,11]. Therefore at most genomic sites methylation patterns are static amongst specific tissues and change in cell-specific context as specialized cellular processes are activated or inactivated. Mechanistically, CpG methylation is thought to mediate gene silencing through direct inhibition of transcription factor binding due to DNA methylation or by recruiting methyl-binding domain proteins that in turn, recruit chromatin-modifying enzymes and complexes to methylated DNA.

Biological regulation of DNA methylation

Most CpG methylations are deposited in a coordinated effort by three conserved enzymes, DNA methyltransferase 1 (DNMT1), DNMT3A, and DNMT3B. During mitosis, DNMT1/UHRF1 recognizes methylated CpGs on the template strand and correspondingly deposits a methyl group on nascent DNA [12]. Although maintenance of DNA methylation at mitosis ensures epigenetic inheritance at most genomic regions, there are many instances in which methylation must be actively targeted and others in which methylation must be inhibited or indirectly removed by exclusion of DNA methyltransferases. For instance, many CpG island promoters are unmethylated, but specific promoters are methylated during development (i.e. de novo methylation) or lineage commitment leading to gene inactivation. In theses cases, DNMT3A and DNMT3B target promoters in complex with other epigenetic repressors, including histone deacetylases (HDACs) and methyltransferases, to remove H3K9 and/or H3K27 methylation and co-operatively repress gene activity [12]. The methylation status of CpG islands also allows for binding of regulatory complexes through specific protein domains. The SRA domain in UHRF1 is able to recognize hemimethylated (mCG:GC) and methyl-CpG-binding domains (MBD) in proteins such as MECP2, MBD1, MBD2, and MBD4 are able to recognize symmetrically methylated (mCG:GCm) CpG dyads [13]. Several zinc finger-CxxC domain proteins including KDM2a (Fbxl11), KDM2b (Fbxl10), and MLL1 specifically recognize non-methylated DNA (CG:GC) and recruit chromatin-modifying activities to CpG islands [13]. Active DNA demethylation of CpG residues is initiated by the TET family of DNA dioxygenases. TET enzymes convert 5-methylcytosine to 5-hydroxymethylcytosine (5hmc) and subsequently 5-formylcytosine (5fC) and 5-carboxycytosine (5caC) leading to nucleotide or base excision repair [9].

DNA methylation in bone

The involvement of DNA methylation in musculoskeletal diseases has been most studied in the context of osteoarthritis. Studies have linked increased methylation at the MMP13 and iNOS promoters in human articular chondrocytes with the progression of arthritic disease [14–16]. Several studies have examined DNA methylation at specific gene promoters as a functional consequence or potential driver of osteoblast differentiation. Differentiation of human mesenchymal stem/stromal cells (MSCs) into the osteoblast lineage resulted in the progressive methylation of promoters associated with pluripotency/stem cell genes [17] or genes involved in ERα signaling [18]. In cells undergoing osteogenic differentiation, changes in the methylation status of the osteoblast-related gene Bglap2 (osteocalcin) gene were observed as a process of mechanical stimulation or differentiation [19,20]. Treatment of MC-3T3-E1 osteoblasts with homocysteine in addition to IL-6 increased levels of DNMT1 and subsequent methylation at specific gene promoters [21]. In the same cell model system, osteoblasts plated on a collagen matrix or treated with DMSO demonstrated increased expression of DNMT1 and TET and an increase in global DNA methylation and methylation of the promoter of the apoptotic gene Fas [22,23]. In a study involving an osteosarcoma model, a DNMT inhibitor reduced DNA methylation and increased expression of osteoblast-related genes such as IL-6, IL6ST, BMP7, ATP6B1, IGF1, WNT1, TNFs and ALPL [24].

Differentiation of osteoblasts from MSCs is an integral part of bone development and homeostasis and as such, the role of DNMTs in maintaining MSC quiescence, senescence and self-renewal has been studied. DNMT1 knockdown in early-passage MSCs induces senescence and reduces differentiation potential through a pRB-mediated pathway, whereas DNMT1 overexpression in late-passage MSCs prevents senescence [25]. Other studies have demonstrated that the inhibition of DNMT1 in MSCs prevents differentiation to osteoblasts, adipocytes or chondrocytes by inducing replicative senescence mediated by p16 and p21 expression [26].

Histone post-translational modifications

Eukaryotic chromatin are assembled into nucleosomes, which are complexes of multiple histone subunits and DNA that act both as physical barrier to DNA access and a source of epigenetic information. Post-translational modifications of histones proteins are a key component of epigenetic regulation influencing lineage commitment and gene expression. Reversible covalent modifications of histones occur at chemically labile residues (i.e. lysine, arginine, serine, threonine, tyrosine and histidine) on accessible N- and C-terminal tail regions and in the less accessible histone fold or globular domains within the nucleosome core [27,28]. Each specific modified residue on the histone protein may relay a specific piece of information. For example, methylation of lysine 4 of histone 3 (H3K4me3), which is a well-studied histone modification, generally indicates that a gene is transcriptionally-poised or active. This information is conferred through multiple mechanisms including altered histone protein–protein or histone protein–DNA interactions or recruitment of binding factors that affect histone modifying and remodeling activities [29]. The most commonly modified histone methylation sites include lysines: H3K4, H3K9, H3K27, H3K36, H3K79 and H4K20 and arginines (R): H3R2, H3R8, H3R17, H3R26 and H4R3. However, several other residues throughout histone H1 and the core histone proteins H2A, H2B, H3 and H4 are modified in various contexts [30]. Although a specific histone modification can act individually and provide information on the expression state of a particular gene, the total of all modifications in a cell represents a comprehensive histone code relating the epigenetic state of a particular cell. This code becomes increasingly complex as particular modifications may act synergistically or antagonistically and the extent or type of modification may vary at individual nucleosomes or individual cells in a population [5]. For example, many promoters in embryonic stem cells are marked by both an H3K4me3 mark and a H3K27me3 mark which would appear conflicting with respect to simple models in which these marks are linked to active and repressed transcriptional states [31]. A large number of histone-modifying enzymes are found in multi-subunit complexes and nearby, modified

Please cite this article as: Gordon JAR, et al, Chromatin modifiers and histone modifications in bone formation, regeneration, and therapeutic intervention for bone-related disea..., Bone (2015), http://dx.doi.org/10.1016/j.bone.2015.03.011