Histone Methylation: Achilles Heel and Powerful Mediator of Periodontal Homeostasis

Journal of Dental Research
2020, Vol. 99(12) 1332–1340
© International & American Associations
for Dental Research 2020
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/0022034520932491
journals.sagepub.com/home/jdr

M. Francis¹, G. Gopinathan², D. Foyle², P. Fallah², M. Gonzalez², X. Luan^{1,2},
and T.G.H. Diekwisch^{1,2}

Abstract

The packaging of DNA around nucleosomes exerts dynamic control over eukaryotic gene expression either by granting access to the transcriptional machinery in an open chromatin state or by silencing transcription via chromatin compaction. Histone methylation modification affects chromatin through the addition of methyl groups to lysine or arginine residues of histones H3 and H4 by means of histone methyl transferases or histone demethylases. Changes in histone methylation state modulate periodontal gene expression and have profound effects on periodontal development, health, and therapy. At the onset of periodontal development, progenitor cell populations such as dental follicle cells are characterized by an open H3K4me3 chromatin mark on RUNX2, MSX2, and DLX5 gene promoters. During further development, periodontal progenitor differentiation undergoes a global switch from the H3K4me3 active methyl mark to the H3K27me3 repressive mark. When compared with dental pulp cells, periodontal neural crest lineage differentiation is characterized by repressive H3K9me3 and H3K27me3 marks on typical dentinogenesis-related genes. Inflammatory conditions as they occur during periodontal disease result in unique histone methylation signatures in affected cell populations, including repressive H3K9me3 and H3K27me3 histone marks on extracellular matrix gene promoters and active H3K4me3 marks on interleukin, defensin, and chemokine gene promoters, facilitating a rapid inflammatory response to microbial pathogens. The inflammation-induced repression of chromatin on extracellular matrix gene promoters presents a therapeutic opportunity for the application of histone methylation inhibitors capable of inhibiting suppressive trimethylation marks. Furthermore, inhibition of chromatin coregulators through interference with key inflammatory mediators such as NF-κB by means of methyltransferase inhibitors provides another avenue to halt the exacerbation of the inflammatory response in periodontal tissues. In conclusion, histone methylation dynamics play an intricate role in the fine-tuning of chromatin states during periodontal development and harbor yet-to-be-realized potential for the treatment of periodontal disease.

Keywords: periodontal disease, chromatin dynamics, small molecule, epigenetic control of transcription, enzymes, inflammation

Introduction

Periodontal disease is an inflammatory condition of the periodontal tissues that affects the DNA of inflamed periodontal cells through epigenetic modifications (Larsson et al. 2015). Epigenetic modifications cause changes in gene expression that do not involve the DNA sequence itself but rather the packaging of DNA around nucleosomes, resulting in changes in chromatin structure. Changes in chromatin structure profoundly affect gene transcription, as they control the accessibility of DNA to transcription factors and the recruitment of RNA polymerase II to the transcription start site (Fig. 1). Within the chromatin, the DNA is wrapped around a core of 8 histones like yarn around a spool, and each histone octamer, including its corresponding segment of DNA, is called a *nucleosome*. Histones have tails protruding from the nucleosome, and especially the tails of the H3 and H4 histones are frequently modified posttranslationally to modulate gene expression by regulating chromatin states or recruiting histone modifiers (Li et al. 2007; Bowman and Poirier 2015). The epigenetic marking system of histone tail modifications (histone code) profoundly affects DNA transcription, with far-reaching

consequences for cell fate decisions and normal and pathologic development (Jenuwein and Allis 2001). The most prominent posttranslational modification to histone proteins include acetylation, methylation, phosphorylation, ubiquitylation, and sumoylation. Histone acetylation and phosphorylation promote open chromatin and enhance transcription, while histone methylation may result in either chromatin compaction or chromatin relaxation, depending on the position of the histone mark (Peterson and Laniel 2004; Bannister and Kouzarides 2011). Histone H3 is primarily acetylated at lysines 9, 14, 18,

¹Department of Oral Biology, College of Dentistry, University of Illinois at Chicago, Chicago, IL, USA

²Department of Periodontics and Center for Craniofacial Research and Diagnosis, Texas A&M University College of Dentistry, Dallas, TX, USA

A supplemental appendix to this article is available online.

Corresponding Author:

T.G.H. Diekwisch, Department of Periodontics and Center for Craniofacial Research and Diagnosis, Texas A&M University College of Dentistry, 3302 Gaston Avenue, Dallas, TX 75246, USA.
Email: diekwisch@tamu.edu

23, and 56 and methylated at arginine 2 and lysines 4, 9, 27, 36, and 79. Histone trimethylation at lysine 4 (H3K4me3) is considered an active mark and is associated with gene expression, while histone trimethylation marks at lysines 9 and 27 (H3K9me3 and H3K27me3) are considered repressive marks associated with the formation of heterochromatic regions (Kimura 2013; Tollefsbol 2016). In the present review, we focus on histone lysine trimethylation as the most versatile epigenetic modification in control of gene expression. Histone methylation derives its broad effect on gene expression through maintaining a delicate balance among H3K4me3, H3K9me3, and H3K27me3 as predominant trimethylation sites, resulting in diverse chromatin architectures associated with discrete transcription states of downstream genes: poised, activated, or repressed (Li and Li 2018). Histone methylation marks are dynamic or stable throughout the life of a cell and even through mitosis and meiosis and are occasionally inherited from parents to children (Greer and Shi 2012).

Periodontal disease is an inflammatory disease triggered by the chronic exposure of the periodontal tissues to oral cavity biofilms. Clinically, the onset of periodontal disease is characterized by erythema of the marginal gingiva as well as bleeding upon probing. This early stage of periodontal disease is called *gingivitis* and is caused by a bacterial invasion that induces the junctional epithelial cells to release cytokines (i.e., interleukins 1 and 6 [IL-1 and IL-6]), which then provoke the small blood vessels to dilate and release more cytokines and polymorphonuclear leukocytes (PMNs; Page 1991). PMNs in turn travel through the connective tissue to the junctional epithelium to phagocytize the bacteria in the periodontal pocket (Page 1991). The increase of PMNs traveling from the blood to the infected site is the host's initial response to the invasive bacteria, known as *acute inflammation* (Bayarsaihan 2011). Thereafter, PMNs start to destroy healthy connective tissue and release matrix metalloproteinases (MMPs) that degrade the extracellular matrix components, including collagen (Page 1991). As the disease progresses, T lymphocytes, B lymphocytes, and eventually plasma cells take over the infected site, and as more tissue is damaged, the tissue destruction overwhelms the tissue repair, resulting in tissue degradation and alveolar bone resorption (Bartold and Van Dyke 2013). At the interface between reparative and destructive processes, individual cells respond with changes in chromatin state that either maintain tissue homeostasis or result in tissue loss. These cellular responses to inflammation are modulated on an epigenetic level (De Santa et al. 2009; Diomedea et al. 2017; Kang et al. 2017). The present review summarizes our current understanding of the events

Figure 1. Epigenetic control of gene expression. Gene expression is controlled by epigenetic factors and mechanisms, including DNA methylation, histone acetylation and methylation, and chromatin remodeling complexes, which affect chromatin higher-order structure. In general, 2 major chromatin states are distinguished: euchromatin and heterochromatin. Heterochromatin (upper half) is characterized by DNA tightly wrapped around nucleosomes, DNA methylation (red balls), H3K9 and K3K27 histone methylation marks, the heterochromatin protein HP1, and inactive transcription. In contrast, euchromatin (lower half) is distinguished by unmethylated CpG islands (white dots), histone acetylation (Ac), and H3K4me3 histone methylation marks. The DNA in euchromatin is only loosely wrapped around DNA, allowing for access to RNA polymerase II (RNAPol II) and transcription factors (TF) and thus facilitating active transcription. Both DNA and histone posttranslational modifications are regulated by enzymes. DNA methylation is facilitated by DNA methyltransferases. Histone acetylation states are modulated by histone deacetylases and histone acetyltransferases. Histone methylation states are modified by histone demethylases and histone methyltransferases such as SUV39H1 or EZH2.

involving histone methylation as they alter the epigenetic response to periodontal disease.

Histone Methylation Mechanisms

Posttranslational histone modifications affect chromatin structure and transcription. The most common posttranslational histone modifications are histone lysine modifications, and H3 is the most extensively modified histone. Lysine methyl modifications can be mono-, di-, or trimethylated, and varying levels of methylation are associated with alterations in transcriptional states. Changes in histone methylation are accomplished by means of specific enzymes that either add methyl groups to histones (histone methyltransferases, “writers”) or remove them (histone lysine demethylases [KDMs], “erasers”; Huang et al. 2015). Histone methyltransferases methylate histones when they become incorporated onto the nucleosomes (Zhang 2001). Two types of enzymes serve as histone lysine demethylases: the amine oxidases, such as LSD1 (lysine-specific histone demethylase 1, KDM1A; Shi et al. 2004), and the JmjC-containing enzymes, named after the jumonji C (JmjC, Japanese for “cruciform”) domain responsible for their demethylase activity (Anand and Marmorstein 2007). The number of

lysine methylation sites known to be associated with transcriptional activation (e.g., H3K4, H3K36, and H3K79) and repression (e.g., H3K9, H3K27, and H4K20) has increased in recent years. In opposite to H3K9me₃, which is frequently associated with heterochromatin, H3K27me₃ often occurs with H3K4me₃ in bivalent domains—for example, in stem cells poised toward differentiation (Bernstein et al. 2006). Among them, H3K4, H3K9, and H3K27 methylation are the best studied histone methylation sites and are known to have an effect in dental tissues (Dangaria et al. 2011; Gopinathan et al. 2013).

The histone code of H3K4, H3K9, and H3K27 methylation is determined by a series of fairly specific enzymes. H3K4 methyltransferases are categorized into SET domain-containing proteins (including mixed-lineage leukaemias [MLLs], Set1A/B, and SET7/9) and unrelated histone methyltransferases (including ASH1, SMYD, PRDM; Zhang and Liu 2015). MLLs and Set1A/B are components of the COMPASS complex (with other subunits: WD repeat domain 5 [WDR5], retinoblastoma-binding protein 5 [RbPB5], and homeotic discs 2 [ASH2]) that mono-, di-, and trimethylate H3K4 (Zhang and Liu 2015). Enzymes that modify H3K9 include the histone methyltransferases KMT1, KMT2H, and KMT8A/D, while EZH2 catalyzes the trimethylation of H3K27 (Zhang and Liu 2015). H3K4 demethylases include LSD/KDM1, which removes methyl groups from either K4me₂ or K4me₁, resulting in K4me₀, as well as JARID/KDM5, which removes methyl groups from K4me₃ or K4me₂, resulting in K4me₁ (Zhang and Liu 2015). Histone demethylases KDM3, JMJD2/KDM4, and KDM7 demethylate H3K9, while the lysine-specific demethylase 6A acts as a histone demethylase for H3K27 (Zhang and Liu 2015).

Histone methyl modifications affect gene transcription by altering chromatin states, either by modulating the interaction between the histone tails of neighboring nucleosomes or by acting as docking sites to specific nonhistone proteins, such as the chromatin repressor HP1, which then affect chromatin activities and properties. It has been suggested that the methyl group increases the positive charge and hydrophobicity of the lysine residue on the histone tails, which is then drawn to interact with the hydrophobic properties of the nonhistone proteins (Greer and Shi 2012). The methylated lysines interact with 1 of 3 protein motifs, including the chromodomain, the tudor domain, and the WD40 repeat domain (Martin and Zhang 2005). Once bound to the methylated residues, these proteins bring together various molecular complexes (Pek et al. 2012).

Histone Methylation and Odontoblastogenesis/Osteoblastogenesis

The periodontium is a tissue characterized by high turnover rates and continuous remodeling. To maintain physiologic alveolar bone levels, mesenchymal stem cells (MSCs) from the periodontal ligament (PDL), periodontal blood vessel pericytes, and alveolar bone progenitors jointly contribute to the renewal of alveolar bone (Pacios et al. 2015; Zhou et al. 2016;

Luan et al. 2017). The continuous recruitment and differentiation of mesenchymal progenitors for the renewal of alveolar bone are essential for periodontal bone homeostasis (Diekwisch 2019; Gopinathan et al. 2019). One of the very first genes involved in alveolar bone osteogenic lineage commitment in the periodontal region is the runt-related transcription factor 2 (Runx2; Luan et al. 2009), and its expression is epigenetically controlled by the histone demethylase JARID1D1B/KDM5B (Rojas et al. 2015). Runx2 is also a common odontogenic lineage marker gene for the 5 committed neural crest-derived dental MSCs: dental follicle (DF), dental pulp (DP), PDL, apical papilla, and alveolar bone progenitor cells (Luan et al. 2009; Dangaria et al. 2011). Epigenetic studies revealed that cells from these committed odontogenic lineages featured H3K4me₃-active marks on the promoters of the early osteogenic lineage transcription factors *RUNX2*, *Msh* homeobox 2 (*MSX2*), and distal-less homeobox 5 (*DLX5*), while mineralization-related gene promoters, including osteonin (*OSX*), integrin-binding sialoprotein (*IBSP*), and bone gamma-carboxyglutamate protein (*BGLAP*) promoters, were enriched for H3K9me₃ or H3K27me₃ repressive marks (Gopinathan et al. 2013). Confirming the specificity of the epigenetic histone methylation signature as a differentiation marker, there was a global epigenetic switch from the euchromatin marker H3K4me₃ to the transcriptional repressor H3K9me₃ when dental progenitors were differentiated under osteogenic induction conditions (Dangaria et al. 2011). This histone methyl mark switch from the active H3K4me₃ mark in progenitors to the H3K9me₃ repressive mark in differentiated cells was confirmed when DF progenitors were compared with differentiated PDL cells, revealing high levels of H3K4me₃ in DF progenitors and high levels of H3K9me₃ in further differentiated PDL cells (Dangaria et al. 2011).

Changes in histone methylation signatures also appear to be at the very heart of odontogenic lineage specification. When asking for the key deciding factor responsible for the fate specification of neural crest cells into DP or DF/PDL progenitors, we identified H3K27me₃ repressive marks on dentin sialophosphoprotein (DSPP) and dentin matrix protein 1 (DMP1) dentinogenesis gene promoters and a lack of DSPP and DMP1 gene expression in DF and PDL cells. In contrast, DP progenitors featured low levels of the H3K27me₃ repressive mark and high levels of DSPP and DMP1 gene expression (Gopinathan et al. 2013). This study suggests that epigenetic repression of the DSPP and DMP1 dentin-specific genes is an essential attribute of periodontal lineage cells, while dentinogenesis lineage cells featured open chromatin on the DSPP and DMP1 promoters. The same study also demonstrated that DF and DP progenitors were marked with the active H3K4me₃ modification on the promoters of early osteogenic lineage transcription factors, such as *RUNX2*, *MSX2*, and *DLX5*, while the osteoblast marker genes *OSX*, *IBSP*, and *BGLAP* featured repressive H3K9me₃ and H3K27me₃ marks on the promoters of these progenitors (Gopinathan et al. 2013). Thus, this study confirmed the presence of open chromatin on osteoblast lineage genes in less differentiated progenitors, while classic osteoblast differentiation

marker genes were further repressed at this stage (Gopinathan et al. 2013).

Together, these studies demonstrated the following. 1) Early mineralized lineage gene promoters in odontogenic progenitors are characterized by open chromatin, while differentiated osteoblast marker gene promoters feature repressive histone marks. 2) Periodontal differentiation from DF to PDL involves a global switch from the H3K4me active mark to the H3K9me3 repressive mark. 3) Epigenetic repression of the DMP1 and DSPP dentin mineralization genes occurs via H3K27 trimethylation in DF progenitors and not in DP cells, suggesting that periodontal lineage differentiation involves an epigenetic “off” switch for dentin-specific genes in neural crest-derived progenitors. 4) In osteogenic progenitor cells, active histone methylation marks occupy the promoters of osteogenic lineage genes, while differentiated osteoblasts feature repressive histone methylation marks on these gene promoters (Gopinathan et al. 2013).

Histone Methylation and Osteoclastogenesis

Periodontal bone homeostasis is regulated by a delicate balance between anabolic and catabolic processes, suggesting that healthy osteoclast physiology is essential for periodontal health (Luan et al. 2017). Osteoclast differentiation is regulated by histone modification conformation dynamics on key developmental genes. A comprehensive ChIP-seq analysis (chromatin immunoprecipitation sequencing) identified 49 genes associated with changes in histone methylation marks from the H3K4me3/H3K27me3 bivalent pattern at the transcription start sites of bone marrow mononuclear cells to the H3K4me3 monovalent pattern in mature osteoclasts, and 33 genes among the 49 genes were nuclear factor of activated T cells 1 (NFATc1) targets (Yasui et al. 2011). One of the genes regulated by NFATc1 that demonstrated a change in histone methylation conformation during osteoclast differentiation was the cell surface adhesion molecule 1 (*Cadm1*), which functions to regulate osteoclastic bone resorption in a negative feedback loop (Nakamura et al. 2017).

Several transcription factors essential for osteoclastogenesis (nuclear factor kappa-light-chain-enhancer of activated B cells 1 and 2 [*NFKB1*, *NFKB2*], Finkel-Biskis-Jenkins osteosarcoma [*FOS*], and *NFATC1*) are labeled by active H3K4me3 marks in bone marrow-derived macrophages and osteoclasts (Yasui et al. 2011). The repressive H3K27me3 mark, however, was detected on only the *NFATC1* gene, and upon treatment with receptor activator of nuclear factor kappa-B ligand (RANKL), the occupancy of this repressive mark decreased while the H3K27me3 demethylase JMJD3 was enriched in the same region (Yasui et al. 2011). Knockdown of the *Jmjd3* gene reduced demethylation of H3K27me3 at the transcription start site of *Nfatc1* and suppressed RANKL-induced osteoclast differentiation, indicating that JMJD3 functions to modulate RANKL-induced osteoclastogenesis (Yasui et al. 2011). In addition, knockdown of KDM3/JMJD1C suppressed NF-KB signaling and osteoclastogenesis (Lee et al. 2019).

RANKL-induced osteoclast differentiation is also regulated via the enrichment of another H3K27me3 methyltransferase, EZH2, on the promoter region of the interferon regulatory factor 8 (*IRF8*; Fang et al. 2016). The *IRF8* gene suppresses osteoclastogenesis partially by inhibiting *NFATC1* expression. Upon treatment with RANKL, EZH2 bound to the promoter region of *IRF8* and deposited the repressive histone mark H3K27me3, thereby suppressing transcription of *IRF8* and enhancing osteoclast differentiation (Fang et al. 2016). In this study, EZH2 inhibition led to an interference in osteoclast differentiation and attenuated bone loss (Fang et al. 2016).

Irregular osteoclast differentiation has also been linked to global histone hypomethylation in critical illness-induced bone loss (Owen et al. 2015). In this study, reduced global H3K9 and K27 methyl marks were linked to an increase in autophagy-inhibiting genes, including huntingtin (*HTT*) and B-cell lymphoma 2 like 1 (*BCL2L1*; Owen et al. 2015). The upregulation of these autophagy-inhibiting genes was associated with the downregulation of several chromatin modification enzymes, including disruptor of telomeric silencing 1 like (*DOT1L*), RPS6KA5, MLL, KAT2A, SUV39H1, and histone deacetylase 1 and 7 (*HDAC1* and *HDAC7*), suggesting a possible link between histone hypomethylation and inadequate autophagy in the osteoclasts of critically ill patients (Owen et al. 2015). While there is little information available about the epigenetic regulation of osteoclastogenesis in periodontal health and disease, it is anticipated that chromatin-remodeling dynamics similar to those that affect nondental bone homeostasis also play a role in the maintenance and resorption of periodontal bone.

Histone Methylation Alteration in Periodontal Disease: Animal Models and Humans

The involvement of epigenetic mechanisms in the inflammatory response to periodontal pathogens opens up therapeutic opportunities for the treatment of periodontal disease (Lindroth and Park 2013; Ari et al. 2016). Evidence for the therapeutic potential of histone methylation modifiers was provided by a study focused on the insulin-like growth binding protein 5 (*IGFBP5*) gene, a part of the insulin-like growth factor axis involved in bone metabolism (Liu et al. 2015). This study not only demonstrated that *IGFBP5* was highly expressed in dental tissue-derived MSCs as compared with nondental stem cells but also verified the importance of *IGFBP5* for MSC osteogenic differentiation and anti-inflammatory effects. Upregulation of *IGFBP5* transcription by repression of K27 methylation on the *IGFBP5* promoter through histone demethylase KDM6B (alternatively termed JMJD3) resulted in enhanced periodontal tissue regeneration and alleviated local inflammation in a swine periodontitis model (Liu et al. 2015), underscoring the potential of histone methylation modifiers for periodontal tissue regeneration. The potential for KDM6B (JMJD3) as a gene expression modifier during inflammation has been confirmed by other studies (de Camargo Pereira et al. 2013).

Figure 2. Comparison of histone methylation profiles between control and periodontitis samples. **(A)** Visualization of ChIP-seq tracks for H3K4me3, H3K9me3, and H3K27me3 enrichment at selected gene promoters in control and periodontitis samples via the IGV genome browser (Integrative Genomics Viewer). The transcription start site for each gene is indicated as a vertical line at the bottom, and the arrowhead marks the direction of transcription on the coding strand. **(B)** Real-time polymerase chain reaction validation of H3K27me3 histone methylation enrichment in control and periodontitis samples. ChIP DNA was amplified with specific primers annealing within the promoter region of the indicated genes. Real-time enrichment data for each primer pair were normalized to the corresponding input. Note the significant increase in enrichment for the repressive K27 mark on matrix-related gene promoters (collagen, cadherin, Mmp2), while K27 enrichment was reduced on inflammation-related gene promoters (Il-6, defensin b-4).

Periodontal ligament degradation and alveolar bone loss indicate that the periodontium has lost its homeostasis capacity under inflammation (Graves et al. 2019). Several studies have shown that PDL stem cells from patients with periodontitis display changes in proliferation and differentiation potential and cell migration capacity (Zhang et al. 2012). Our ChIP-on-chip analysis demonstrated that H3K27me3 enrichment for extracellular matrix genes such as collagen I, cadherin I, and MMP2 increased in our mouse periodontitis models (Fig. 2A, B), while H3K27 was reduced on IL-6 and defensin b-4 inflammatory gene promoters, suggesting a more open chromatin and more active transcriptional state on those promoters. These findings match earlier studies from our laboratory also reporting a global reduction in H3K27me3 enrichment on inflammation response gene promoters (Francis et al. 2019). As mentioned, H3K27me3 is a bivalent chromatin mark—a mark that is most easily affected by dynamic changes in levels of inflammation. Here our data suggest that H3K27me3 modulates the inflammatory response to periodontal pathogens by opening the chromatin on

inflammatory gene promoters and by repressing the chromatin on extracellular matrix gene promoters. Our gene ontology studies based on ChIP-seq data (Fig. 3) confirm the dramatic increase in the number of peaks in the periodontitis H3K27me3 group compared with the control H3K27me3 group (Fig. 3A) and an increase in proximity to the transcription start site for the H3K27me3 histone methylation mark (Fig. 3C) in our periodontitis group versus control, confirming the functional significance of the H3K27me3 mark in response to inflammation.

Targeting Histone Methylation as a Strategy for the Treatment of Periodontal Disease

Periodontal disease, inflammation, and chromatin state are closely linked: a relationship that not only contributes to pathogenesis but also harbors inherent opportunities for the treatment of periodontal disease or for the development of novel markers of disease progression. As an example, periodontal

tissues of individuals with periodontitis demonstrated elevated levels of inflammatory cytokines, open chromatin on inflammatory cytokine gene promoters, and epigenetic marks indicative of active transcription, including histone H3 lysine 4 trimethylation and high levels of histone H3 and H4 acetylation at various lysine residues (Gomez et al. 2009). Upon treatment or in healthy patients, the chromatin at the promoters of inflammatory cytokines becomes compacted. However, this process may easily become reversed once the bacteria of the oral cavity interact with toll-like receptors (Barros and Offenbacher 2014).

The hallmark of periodontal disease is the reduced osteogenic differentiation potential of PDL progenitors in patients or animals with periodontitis (Liu et al. 2009; Liu et al. 2011). Periodontal ligament cells from such patients or animals display osteogenesis defects in vitro due to the changes in epigenetic profiles as a result of the periodontal disease (Liu et al. 2009; Sun et al. 2010; Liu et al. 2011). However, the inherent plasticity of epigenetic dysregulation offers potential opportunities for the treatment of periodontal disease by using specific modulators to target histone modification enzymes or proteins maintaining histone methylation balance. Here we review a number of epigenetic modulators that either hold direct potential for the treatment of periodontal disease or illustrate the mode of action that might prove useful in the future once adequate modulators have been identified (Table).

DZNeps are powerful histone methylation inhibitors, as they reactivate developmental genes by inhibiting suppressive trimethylation marks (Sarkar et al. 2014). 3-Deazaneplanocin A (DZNep) specifically functions as an inhibitor of S-adenosyl-L-homocysteine hydrolase (SAHH) and affects tissue regeneration by inhibiting EZH2 (Miranda et al. 2009; Girard et al. 2014). Whole-transcriptome analysis demonstrated that DZNep counteracts the effect of IL-1 β on the expression of 81 protein-coding genes, including *CITED2*, an MMP inhibitor. Its effect on IL-1 β inhibition, reversal of EZH2, and resulting increase in bone formation may prove useful in the treatment of periodontal disease.

Protein methyltransferase inhibitors are another class of epigenetic drugs used to block the methylation of lysines and arginines (Sarkar et al. 2014). Chaetocin, a fungal mycotoxin, inhibits Su(var)3-9 (a histone methyltransferase for H3K9me3) and G9a (a histone methyltransferase for H3K9me1 and H3K9me2; Bissinger et al. 2010). BIX-01294 also inhibits G9a and is selective against SUV39H1 and PRMT1. The structure of BIX-01294 has been modified to produce UNC0224, a new inhibitor for G9a with a 1000-fold selectivity for G9a as

Figure 3. ChIP-seq enrichment peak distribution and annotation with HOMER. **(A)** Comparison of peak count based on total number of enrichment peaks called by MACS2 for H3K4me3, H3K9me3, and H3K27me3 histone modifications in control and periodontitis samples. Note the massive increase in the number of peaks in the periodontitis H3K27me3 group as compared with the control H3K27me3 group. **(B)** Enrichment peaks corresponding to H3K4me3, H3K9me3, and H3K27me3 histone modifications in control and periodontitis samples were annotated by calculating the proportion of peaks assigned to individual genomic locations (exon, intergenic, intron, promoter-TSS, and TTS [transcription termination site]). **(C)** Distribution of enrichment peaks relative to the TSS as visualized by violin plots. The vertical rectangular box for each data point indicates the interquartile range, and the horizontal line within the box marks the median data point for the distribution. The probability density and distribution shape of data points were visualized on both sides of the box plot. ChIP-seq, chromatin immunoprecipitation sequencing.

compared with SET7/9 and Set8 (Bissinger et al. 2010), possibly reducing side effects of BIX-01294.

Lysine demethylase inhibitors may also hold potential as a drug for the treatment of periodontal lesions due to their inhibitory effect on fibrosis and their potential to restore extracellular matrix environments. As one study in collagenase-induced osteoarthritic knees in mice demonstrated, intra-articular administration of the KDM6a inhibitor GSK-J4 dependently improved fibrotic matrix accumulation and subchondral bone microarchitecture deterioration in injured joints. Treatment with GSK-J4 decreased expression of fibrogenic factors such as TGF- β 1, procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 (PLOD2), and tissue inhibitor of metalloproteinase (TIMP) and restored the expression of cartilage and bone matrices (collagen II and I, aggrecan, and osteocalcin) by inhibiting KDM6a and reversing SRY-box transcription factor (SOX9) mRNA

Table. Opportunities and Limitations of Small Molecule Epigenetic Inhibitors as Strategies for the Treatment of Periodontal Disease.

Name	Mechanism of Action	Type of Molecule	Functional Effects	Limitations
DZNep	EZH2 inhibitor	Small molecule	Counteracts the effect of IL-1 β and increases expression of matrix metalloproteinases (MMP-2 and MMP-9), as well as the phosphorylation of Raf-1 and ERK1/2 (Aury-Landas et al. 2017; Zhou et al. 2018)	Lack of specificity
Chaetocin	SUV39H1, G9a	Small molecule	Fungal metabolite known for its anticancer activities. Functions by inhibiting lysine-specific histone methyltransferases. Upregulates E-cadherin and P15INK4B (Greiner et al. 2005; Lakshmikuttyamma et al. 2010).	Impairs proliferation and induces apoptosis by regulating ASK-1/JNK signaling
BIX-01294	Inhibitor of G9a, SUV39H1, blocks histone-binding sites	Small molecule	Downregulates proliferation and migration genes as well as MMP2 expression; upregulates IL-6, GAD67, NANOG, and KLF4 (Oh et al. 2015; Chase et al. 2019)	Exacerbates foam cell formation
GSK-J4	KDM/JMJD3 inhibitor; increases H3K27me3 global levels	Small molecule	Decreases cell proliferation and migration. Inhibits inflammatory phenotypes of natural killer cells. Promotes ossification-related gene expression (Kruidenier et al. 2012; Cribbs et al. 2018; Sun et al. 2018)	Proliferation inhibitor
GSK-126	EZH2 inhibitor	Small molecule	Promotes expression of bone-related gene regulators and extracellular matrix proteins. Prevents fibrosis (Dudakovic 2016; Song et al. 2019)	Changes in bone homeostasis, reprogramming of stem cells
IGFBP5	Downstream effector of KDM6B	Protein	Anti-inflammatory effects. Enhanced osteogenic differentiation and periodontal regeneration (Liu et al. 2015).	Promotes fibrosis

and protein levels in joint tissues (Sun et al. 2018). In contrast, inhibition of KDM4B in osteoclast progenitors by the KDM4B/JMMD2 demethylase inhibitor ML324 attenuated lipopolysaccharide-induced proinflammatory cytokine expression and osteoclastogenesis (Kirkpatrick et al. 2018).

EZH2 is the catalytic subunit of polycomb repressive complex 2 (PRC2), which is a highly conserved histone methyltransferase that decreases lysine-27 H3 trimethylation (Simon and Lange, 2008). EZH2 contributes to stem cell maintenance and cell fate decision during cell lineage specification (Chou et al. 2015) and may also play a role in DP regeneration (Hui et al. 2017). Pharmacologic inhibition via GSK126 and siRNA knockdown of EZH2 enhanced osteogenic commitment of MC3T3 preosteoblasts (Dudakovic et al. 2016). Next-generation RNA sequencing of mRNAs and real-time quantitative polymerase chain reaction profiling established that Ezh2 inactivation promotes expression of bone-related gene regulators and extracellular matrix proteins. Administration of an Ezh2 inhibitor modestly increases bone density in adult mice (Dudakovic et al. 2016). Ezh2 inhibition enhanced expression of Wnt10b and Pth1r and increased the BMP-dependent phosphorylation of Smad1/5. Thus, these data suggest that inhibition of Ezh2 promotes paracrine signaling in osteoblasts and has bone-anabolic and osteoprotective potential in adults (Dudakovic et al. 2016), suggestive of a beneficial role in the treatment of periodontal disease.

Original studies from our laboratory indicated that insulin growth factor-binding protein 5 (IGFBP5) was highly expressed in periodontal tissues and alveolar bone (Dangaria et al. 2011). However, IGFBP5 was downregulated in periodontal tissues of individuals with periodontitis as

compared with healthy controls (Liu et al. 2015). In this study, depletion of the lysine (K)-specific demethylase 6B (KDM6B) downregulated IGFBP5 expression by increasing histone K27 methylation on the IGFBP5 promoter. Moreover, IGFBP5 treatment resulted in enhanced osteogenic differentiation and improved the anti-inflammatory potential of MSCs by inhibiting NF- κ B signaling. Illustrating its potential as drug employing epigenetic mechanisms for the treatment of periodontal disease, IGFBP5 enhanced MSC-mediated periodontal tissue regeneration and alleviated local inflammation in a swine model of periodontitis (Liu et al. 2015).

Together, these studies indicate that epigenetic modifiers exert powerful control over periodontal inflammation and that their application halts and reverses some of the detrimental tissue destruction associated with periodontal disease. Due to their effect on chromatin packaging, drug-induced epigenetic modifications may be associated with increasing genomic instability (Purrucker and Mahlknecht 2010). However, local delivery in tailored scaffolds through controlled release will limit systemic side effects due to their ability to control final tissue concentrations (Jahangirian et al. 2019). Furthermore, improvements in linker technology and conjugation chemistries and recent advances in bioorthogonal chemistry will avail complexed small molecule therapeutics in an on-demand fashion and rapidly close the gap toward human application (Devaraj 2018; Peplow 2019). Recent evidence demonstrates that periodontal disease alone causes genome instability (Borba et al. 2019), and titanium particles, as they shed from periodontal implants, have been linked to genome instability as well (Coen et al. 2003; Ponsford and Diekwisch 2020). Only further advances in periodontal research and precision dentistry will allow for an

informed assessment of the risk-to-benefit ratios of surgical versus conservative versus drug-based periodontal therapies.

During periodontal disease, the opening of chromatin on inflammatory gene promoters is arguably the most dramatic response to periodontal pathogens: an effective shunt for the host response and an Achilles heel for periodontal tissue integrity at the same time. Yet, the responsiveness of the chromatin to changes in histone methylation state harbors unforeseen therapeutic opportunities involving novel small molecule mediators that selectively alter chromatin dynamics and benefit periodontal health.

Author Contributions

M. Francis, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; G. Gopinathan, contributed to design, data analysis, and interpretation, drafted and critically revised the manuscript; D. Foyle, P. Fallah, contributed to conception and data interpretation, drafted and critically revised the manuscript; M. Gonzalez, contributed to design and data interpretation, drafted and critically revised the manuscript; X. Luan, contributed to conception, design, and data acquisition, drafted and critically revised the manuscript; T.G.H. Diekwisch, contributed to conception, design, data acquisition, and analysis, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

Acknowledgments

Generous funding for these studies was provided by National Institute of Dental and Craniofacial Research grants DE026198 and DE027930 to T.G.H.D., DE019463 to X.L., and F30 DE 024352 to M.F. The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

ORCID iD

T.G.H. Diekwisch <https://orcid.org/0000-0003-3356-9677>

References

- Anand R, Marmorstein R. 2007. Structure and mechanism of lysine-specific demethylase enzymes. *J Biol Chem*. 282(49):35425–35429.
- Ari G, Cherukuri S, Namasivayam A. 2016. Epigenetics and periodontitis: a contemporary review. *J Clin Diagn Res*. 10(11):ZE07–ZE09.
- Aury-Landas J, Bazille C, Allas L, Bouhout S, Chesneau C, Leclercq S, Boumédiène K, Baugé C. 2017. Anti-inflammatory and chondroprotective effects of the S-adenosylhomocysteine hydrolase inhibitor 3-deazaneplanocin A, in human articular chondrocytes. *Sci Rep*. 7(1):6483.
- Bannister AJ, Kouzarides T. 2011. Regulation of chromatin by histone modifications. *Cell Res*. 21(3):381–395.
- Barros SP, Offenbacher S. 2014. Modifiable risk factors in periodontal disease: epigenetic regulation of gene expression in the inflammatory response. *Periodontol* 2000. 64(1):95–110.
- Bartold PM, Van Dyke TE. 2013. Periodontitis: a host-mediated disruption of microbial homeostasis. *Unlearning learned concepts*. *Periodontol* 2000. 62(1):203–217.
- Bayarsaihan D. 2011. Epigenetic mechanisms in inflammation. *J Dent Res*. 90(1):9–17.
- Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, et al. 2006. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell*. 125(2):315–326.
- Bissinger EM, Heinke R, Sippl W, Jung M. 2010. Targeting epigenetic modifiers: inhibitors of histone methyltransferases. *MedChemComm*. 1(2):114–124.
- Borba TT, Molz P, Schlickmann DS, Santos C, Oliveira CF, Prá D, Neto LK, Franke SIR. 2019. Periodontitis: genomic instability implications and associated risk factors. *Mutat Res Genet Toxicol Environ Mutagen*. 840:20–23.
- Bowman GD, Poirier MG. 2015. Post-translational modifications of histones that influence nucleosome dynamics. *Chem Rev*. 115(6):2274–2295.
- Chase KA, Feiner B, Ramaker MJ, Hu E, Rosen C, Sharma RP. 2019. Examining the effects of the histone methyltransferase inhibitor BIX-01294 on histone modifications and gene expression in both a clinical population and mouse models. *PLoS One*. 14(6):e0216463.
- Chou RH, Chiu L, Yu YL, Shyu WC. 2015. The potential roles of EZH2 in regenerative medicine. *Cell Transplant*. 24(3):313–317.
- Cribbs A, Hookway ES, Wells G, Lindow M, Obad S, Oerum H, Prinjha RK, Athanasou N, Sowman A, Philpott M, et al. 2018. Inhibition of histone H3K27 demethylases selectively modulates inflammatory phenotypes of natural killer cells. *J Biol Chem*. 293(7):2422–2437.
- Coen N, Kadhim MA, Wright EG, Case CP, Mothersill CE. 2003. Particulate debris from a titanium metal prosthesis induces genomic instability in primary human fibroblast cells. *Br J Cancer*. 88(4):548–552.
- Dangaria SJ, Ito Y, Luan X, Diekwisch TG. 2011. Differentiation of neural-crest-derived intermediate pluripotent progenitors into committed periodontal populations involves unique molecular signature changes, cohort shifts, and epigenetic modifications. *Stem Cells Dev*. 20(1):39–52.
- de Camargo Pereira G, Guimarães GN, Planello AC, Santamaria MP, de Souza AP, Line SR, Marques MR. 2013. *Porphyromonas gingivalis* LPS stimulation downregulates DNMT1, DNMT3a, and JMJD3 gene expression levels in human HaCaT keratinocytes. *Clin Oral Investig*. 17(4):1279–1285.
- De Santa F, Narang V, Yap ZH, Tusi BK, Burgold T, Austenaa L, Buccì G, Caganova M, Notarbartolo S, Casola S, et al. 2009. Jmjd3 contributes to the control of gene expression in LPS-activated macrophages. *EMBO J*. 28:3341–3352.
- Devaraj NK. 2018. The future of bioorthogonal chemistry. *ACS Cent Sci*. 4(8):952–959.
- Diekwisch TGH. 2019. Periodontal homeostasis: from Vienna to Texas—a century of periodontal research in the spirit of Bernhard Gottlieb. *Stem Cells Dev*. 28(15):961–962.
- Diomedea F, Thangavelu SR, Merciaro I, D'Orazio M, Bramanti P, Mazzon E, Trubiani O. 2017. *Porphyromonas gingivalis* lipopolysaccharide stimulation in human periodontal ligament stem cells: role of epigenetic modifications to the inflammation. *Eur J Histochem*. 61(3):2826.
- Dudakovic A, Camilleri ET, Riester SM, Paradise CR, Gluscevic M, O'Toole TM, Thaler R, Evans JM, Yan H, Subramaniam M, et al. 2016. Enhancer of zeste homolog 2 inhibition stimulates bone formation and mitigates bone loss caused by ovariectomy in skeletally mature mice. *J Biol Chem*. 291(47):24594–24606.
- Fang C, Qiao Y, Mun SH, Lee MJ, Murata K, Bae S, Zhao B, Park-Min KH, Ivashkiv LB. 2016. Cutting edge: EZH2 promotes osteoclastogenesis by epigenetic silencing of the negative regulator IRF8. *J Immunol*. 196(11):4452–4456.
- Francis M, Pandya M, Gopinathan G, Lyu H, Ma W, Foyle D, Nares S, Luan X. 2019. Histone methylation mechanisms modulate the inflammatory response of periodontal ligament progenitors. *Stem Cells Dev*. 28(15):1015–1025.
- Girard N, Bazille C, Lhuissier E, Benateau H, Llombart-Bosch A, Boumediene K, Bauge C. 2014. 3-Deazaneplanocin A (DZNep), an inhibitor of the histone methyltransferase EZH2, induces apoptosis and reduces cell migration in chondrosarcoma cells. *PLoS One*. 9(5):e98176.
- Gomez RS, Dutra WO, Moreira PR. 2009. Epigenetics and periodontal disease: future perspectives. *Inflamm Res*. 58(10):625–629.
- Gopinathan G, Foyle D, Luan X, Diekwisch TGH. 2019. The Wnt antagonist SFRP1: a key regulator of periodontal mineral homeostasis. *Stem Cells Dev*. 28(15):1004–1014.
- Gopinathan G, Kolokythas A, Luan X, Diekwisch TGH. 2013. Epigenetic marks define the lineage and differentiation potential of two distinct neural crest-derived intermediate odontogenic progenitor populations. *Stem Cells Dev*. 22(12):1763–1778.
- Graves DT, Ding Z, Yang Y. 2019. The impact of diabetes on periodontal diseases. *Periodontol* 2000. 8(1):214–224.
- Greer EL, Shi Y. 2012. Histone methylation: a dynamic mark in health, disease and inheritance. *Nat Rev Genet*. 13(5):343–357.
- Greiner D, Bonaldi T, Eskeland R, Roemer E, Imhof A. 2005. Identification of a specific inhibitor of the histone methyltransferase SU(VAR)3-9. *Nat Chem Biol*. 1(3):143–145.
- Huang B, Li G, Jiang XH. 2015. Fate determination in mesenchymal stem cells: a perspective from histone-modifying enzymes. *Stem Cell Res Ther*. 6:35.
- Hui T, Wang C, Chen D, Zheng L, Huang D, Ye L. 2017. Epigenetic regulation in dental pulp inflammation. *Oral Dis*. 23(1):22–28.

- Jahangirian H, Azizi S, Rafiee-Moghaddam R, Baratvand B, Webster TJ. 2019. Status of plant protein-based green scaffolds for regenerative medicine applications. *Biomolecules*. 9(10):E619.
- Jenuwein T, Allis CD. 2001. Translating the histone code. *Science*. 293(5532):1074–1080.
- Kang MK, Mehrzarin S, Park NH, Wang CY. 2017. Epigenetic gene regulation by histone demethylases: emerging role in oncogenesis and inflammation. *Oral Dis*. 23(6):709–720.
- Kimura H. 2013. Histone modifications for human epigenome analysis. *J Hum Genet*. 58(7):439–445.
- Kirkpatrick JE, Kirkwood KL, Woster PM. 2018. Inhibition of the histone demethylase KDM4B leads to activation of KDM1A, attenuates bacterial-induced pro-inflammatory cytokine release, and reduces osteoclastogenesis. *Epigenetics*. 13(5):557–572.
- Kruidenier L, Chung C, Cheng Z, Liddle J, Che K, Joberty G, Bantscheff M, Bountra C, Bridges A, Diallo H, et al. 2012. A selective jumoni H3K27 demethylase inhibitor modulates the proinflammatory macrophage response. *Nature*. 488(7411):404–408.
- Lakshmikuttyamma A, Scott SA, DeCoteau JF, Geyer CR. 2010. Reexpression of epigenetically silenced AML tumor suppressor genes by SUV39H1 inhibition. *Oncogene*. 29(4):576–588.
- Larsson L, Castilho RM, Giannobile WV. 2015. Epigenetics and its role in periodontal diseases: a state-of-the-art review. *J Periodontol*. 86(4):556–568.
- Lee JY, Mehrzarin S, Alshaikh A, Kim S, Chen W, Lux R, Gwak Y, Kim RH, Kang MK. 2019. Histone Lys demethylase KDM3C demonstrates anti-inflammatory effects by suppressing NFκB signaling and osteoclastogenesis. *FASEB J*. 33(9):10515–10527.
- Li B, Carey M, Workman JL. 2007. The role of chromatin during transcription. *Cell*. 128(4):707–719.
- Li XM, Li XD. 2018. Interrogating interactions and modifications of histones in live cells. *Cell Chem Biol*. 25(1):1–3.
- Lindroth AM, Park YJ. 2013. Epigenetic biomarkers: a step forward for understanding periodontitis. *J Periodontol Implant Sci*. 43(3):111–120.
- Liu D, Wang Y, Jia Z, Wang L, Wang J, Yang D, Song J, Wang S, Fan Z. 2015. Demethylation of IGFBP5 by histone demethylase KDM6B promotes mesenchymal stem cell-mediated periodontal tissue regeneration by enhancing osteogenic differentiation and anti-inflammation potentials; IGFBP5 promotes MSC-mediated tissue regeneration. *Stem Cells*. 33(8):2523–2536.
- Liu L, Ling J, Wei X, Wu L, Xiao Y. 2009. Stem cell regulatory gene expression in human adult dental pulp and periodontal ligament cells undergoing odontogenic/osteogenic differentiation. *J Endod*. 35(10):1368–1376.
- Liu Y, Liu W, Hu C, Xue Z, Wang G, Ding B, Luo H, Tang L, Kong X, Chen X, et al. 2011. MiR-17 modulates osteogenic differentiation through a coherent feed-forward loop in mesenchymal stem cells isolated from periodontal ligaments of patients with periodontitis. *Stem Cells*. 29(11):1804–1816.
- Luan X, Dangaria S, Ito Y, Walker CG, Jin T, Schmidt MK, Galang MT, Druzinsky R. 2009. Neural crest lineage segregation: a blueprint for periodontal regeneration. *J Dent Res*. 88(9):781–791.
- Luan X, Zhou X, Trombetta-eSilva J, Francis M, Gaharwar AK, Atsawasuwan P, Diekwisch TGH. 2017. MicroRNAs and periodontal homeostasis. *J Dent Res*. 96(5):491–500.
- Martin C, Zhang Y. 2005. The diverse functions of histone lysine methylation. *Nat Rev Mol Cell Biol*. 6(11):838–849.
- Miranda TB, Cortez CC, Yoo CB, Liang G, Abe M, Kelly TK, Marquez VE, Jones PA. 2009. DN3ep is a global histone methylation inhibitor that reactivates developmental genes not silenced by DNA methylation. *Mol Cancer Ther*. 8(6):1579–1588.
- Nakamura S, Koyama T, Izawa N, Nomura S, Fujita T, Omata Y, Minami T, Matsumoto M, Nakamura M, Fujita-Jimbo E, et al. 2017. Negative feedback loop of bone resorption by NFATc1-dependent induction of Cadm1. *PLoS One*. 12(4):e0175632.
- Oh SY, Seok JY, Choi YS, Lee SH, Bae JS, Lee YM. 2015. The Histone Methyltransferase Inhibitor BIX01294 Inhibits HIF-1α Stability and Angiogenesis. *Mol Cells*. 38(6): 528–534.
- Owen HC, Vanhees I, Gunst J, Van Cromphaut S, Van den Berghe G. 2015. Critical illness-induced bone loss is related to deficient autophagy and histone hypomethylation. *Intensive Care Med Exp*. 3(1):52.
- Pacios S, Xiao W, Mattos M, Lim J, Tarapore RS, Alsadun S, Yu B, Wang CY, Graves DT. 2015. Osteoblast lineage cells play an essential role in periodontal bone loss through activation of nuclear factor-κB. *Sci Rep*. 5:16694.
- Page RC. 1991. The role of inflammatory mediators in the pathogenesis of periodontal disease. *J Periodontol Res*. 26(3):230–242.
- Pek JW, Anand A, Kai T. 2012. Tudor domain proteins in development. *Development*. 139(13):2255–2266.
- Peplow M. 2019. A conversation with Kenichiro Itami. *ACS Cent Sci*. 5(1):1–2.
- Peterson CL, Laniel MA. 2004. Histones and histone modifications. *Curr Biol*. 14(14):R546–R551.
- Ponsford M, Diekwisch TGH. 2020. Peri-implant disease. In: Sahingur SE, editor. *Emerging therapies in periodontics*. Cham (Switzerland): Springer Nature Switzerland AG.
- Purruccer JC, Mahlknecht U. 2010. Targeting the epigenome: effects of epigenetic treatment strategies on genomic stability in healthy human cells. *Clin Epigenetics*. 1(1–2):45–54.
- Rojas A, Aguilar R, Henriquez B, Lian JB, Stein JL, Stein GS, van Wijnen AJ, van Zundert B, Allende ML, Montecino M. 2015. Epigenetic control of the bone-master Runx2 gene during osteoblast-lineage commitment by the histone demethylase JARID1B/KDM5B. *J Biol Chem*. 290(47):28329–28342.
- Sarkar S, Heerboth S, Lapinska K, Snyder N, Rollinson S, Leary M. 2014. Use of epigenetic drugs in disease: an overview. *Genet Epigenet*. 6:9–19.
- Shi Y, Lan F, Matson C, Mulligan P, Whetstone JR, Cole PA, Casero RA, Shi Y. 2004. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell*. 119(7):941–953.
- Simon JA, Lange CA. 2008. Roles of the EZH2 histone methyltransferase in cancer epigenetics. *Mutat Res Mol Mech Mutagen*. 647(1–2):21–29.
- Song S, Zhang R, Mo B, Chen L, Liu L, Yu Y, Cao W, Fang G, Wan Y, Gu Y, et al. 2019. EZH2 as a novel therapeutic target for atrial fibrosis and atrial fibrillation. *J Mol Cell Cardiol*. 135:119–133.
- Sun G, Reddy MA, Yuan H, Lanting L, Kato M, Natarajan R. 2010. Epigenetic histone methylation modulates fibrotic gene expression. *J Am Soc Nephrol*. 21(12):2069–2080.
- Sun YC, Chen YS, Wang FS. 2018. Histone lysine demethylase KDM6A inhibitor alleviates osteoarthritis [abstract]. *Orthop Proc*. 99B:Supp 8.
- Tollefsbol T. 2016. *Medical epigenetics*. Cambridge (MA): Academic Press.
- Yasui T, Hirose J, Aburatani H, Tanaka S. 2011. Epigenetic regulation of osteoclast differentiation. *Ann N Y Acad Sci*. 1240(1):7–13.
- Zhang J, An Y, Gao LN, Zhang YJ, Jin Y, Chen FM. 2012. The effect of aging on the pluripotential capacity and regenerative potential of human periodontal ligament stem cells. *Biomaterials*. 33(29):6974–6986.
- Zhang QJ, Liu ZP. 2015. Histone methylations in heart development, congenital and adult heart diseases. *Epigenomics*. 7(2):321–330.
- Zhang Y. 2001. Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails. *Genes Dev*. 15(18):2343–2360.
- Zhou X, Luan X, Chen Z, Francis M, Gopinathan G, Li W, Lu X, Li S, Wu C, Diekwisch TG. 2016. MicroRNA-138 inhibits periodontal progenitor differentiation under inflammatory conditions. *J Dent Res*. 95(2):230–237.
- Zhou X, Zang X, Guan Y, Tolbert T, Zhao TC, Bayliss G, Zhuang S. 2018. Targeting enhancer of zeste homolog 2 protects against acute kidney injury. *Cell Death Dis*. 9(11):1067.