D. Bayarsaihan

Center for Regenerative Medicine and Skeletal Development,
Department of Reconstructive Sciences, School of Dentistry,
University of Connecticut Health Center, 262 Farmington
Avenue, Farmington, CT 06030, USA; dashzeveg@uchc.edu

J Dent Res 90(1):9-17, 2011

ABSTRACT

Epigenetic modifications occur in response to environmental changes and play a fundamental role in gene expression following environmental stimuli. Major epigenetic events include methylation and acetylation of histones and regulatory factors, DNA methylation, and small non-coding RNAs. Diet, pollution, infections, and other environmental factors have profound effects on epigenetic modifications and trigger susceptibility to diseases. Despite a growing body of literature addressing the role of the environment on gene expression, very little is known about the epigenetic pathways involved in the modulation of inflammatory and anti-inflammatory genes. This review summarizes the current knowledge about epigenetic control mechanisms during the inflammatory response.

KEY WORDS: epigenetics, histone modifications, DNA methylation, inflammation.

Epigenetic Mechanisms in Inflammation

INTRODUCTION

Epigenetics is defined as the study of mitotically and meiotically heritable changes in gene function that are not dependent on DNA sequence (Feinberg, 2007). The molecular basis of epigenetic processes is complex and involves modifications of histones, methylation of DNA, positioning of histone variants, and gene regulation by non-coding RNAs. Epigenetic modifications are potentially reversible, and, therefore, a thorough understanding of these changes may identify new therapeutic targets for disease.

The epigenome, the overall epigenetic state of an organism, is just as important as the genome to normal development. Importantly, environmental factors (nutrients, toxins, infections, hypoxia) can have profound effects on the epigenetic signature (Fig. 1) and trigger susceptibility to disease (Barros and Offenbacher, 2009; Safronova and Morita, 2010). For example, recent studies have shown that the fetal environment can cause changes in the epigenome, with long-term consequences for gene regulation and age-related diseases (Thompson and Einstein, 2010). The studies by Bobetsis *et al.* (2006) showed that periodontal infection can lead to placental-fetal exposure and, when coupled with a fetal inflammatory response, leads to preterm delivery.

INFLAMMATION

Inflammation is a complex physiological response of an organism to harmful stimuli, such as pathogens, damaged cells, or irritants. In acute inflammation, the initial response of the body to a stimulus is achieved by increasing the migration of leukocytes and plasma from the blood to the injured areas. When inflammation has a slow onset and persists for a long period of time, it becomes chronic. The symptoms in chronic inflammation are not as severe as in acute inflammation, but the condition is persistent. Chronic inflammation underlies many diseases, including periodontal disease and diabetes mellitus (Dunning, 2009).

The complexity of the inflammatory response requires the development of a sophisticated regulatory network to carry out functions at signal-specific and gene-specific levels (Medzhitov and Horng, 2009). This network involves the activation of specific genes for antimicrobial defense, immune response, and tissue repair and remodeling (Medzhitov, 2008). Macrophages play critical roles in diverse chronic diseases, including cancer and allergic responses, and analysis of recent data indicates that chromatin modifications are mechanistically important in the acquisition of the macrophage phenotype (Khansari *et al.*, 2009). Transcription factors of the NF- κ B, FOXP3, IRF, and STAT families along with epigenetic phenomena, including DNA methylation and covalent histone modifications, have been shown to be critical in the regulation of inflammatory genes (Medzhitov and Horng, 2009). In addition, several of these regulatory factors are controlled by epigenetic mechanisms in T-cells and monocytes (Lal *et al.*, 2009; Wells, 2009; Wierda *et al.*, 2010).

DOI: 10.1177/0022034510378683

Received March 11, 2009; Last revision June 23, 2010;
Accepted June 23, 2010

© International & American Associations for Dental Research

Figure 1. Environment and the epigenome. Differential expression of genes is dependent on chromatin organization. This organization is composed of DNA, nucleosomes, non-histone proteins, transcription factors, chromatin-modifying enzymes, and regulatory RNAs collectively known as the epigenome. The epigenome is sensitive to stress, toxins, nutrition, infections, and other environmental factors with long-term consequences for gene regulation and age-related diseases.

HISTONE MODIFICATIONS

The basic unit of chromatin, the nucleosome, consists of a short segment of DNA wrapped around core histones made up of two copies of H2A, H2B, H3, and H4. This organization provides a rigid structure to chromatin (Campos and Reinberg, 2009). The covalent modification of histones is an essential epigenetic mechanism of gene regulation. These post-translational modifications (methylation of lysines and arginines, acetylation, phosphorylation, ubiquitination, SUMOylation, and ADP-ribosylation) occur most frequently at the N-terminal tails of the core histones (Fuchs *et al.*, 2006).

Acetylation of histones is associated with an “open” chromatin conformation that facilitates transcription (Campos and Reinberg, 2009; Cheng and Blumenthal, 2010). The acetylated N termini protruding from the nucleosome core provide reduced affinity for the DNA, allowing the chromatin to adopt a more relaxed structure for the recruitment of the basic transcription machinery. For example, the acetylated histone marks H3K4ac and H3K39ac are associated with transcriptional activation. Histone acetyltransferases (HATs) such as CREB-binding protein CBP and its close homolog p300 carry out these modifications (Timmermann *et al.*, 2001). Histone deacetylases (HDACs) reverse HAT activity by making chromatin more condensed and

by promoting gene repression. Histone methylation, in contrast, can keep chromatin in either an activated or a repressed state. Tri-methylation of histone H3 on lysine 4 and 36 (H3K4me3 and H3K36me3) facilitates an open chromatin for active transcription (Barski *et al.*, 2007). By contrast, histone methylations on lysines 9 and 27 (H3K9me3 and H3K27me3) are generally associated with a condensed chromatin and gene silencing (Lan and Shi, 2009). Genes with bivalent modification (H3K4me3 and H3K27me3) are typically important developmental regulators in pluripotent embryonic stem (ES) cells or multipotent progenitor cells which are silenced, but are poised for activation, as differentiation proceeds (Bernstein *et al.*, 2006; Mikkelsen *et al.*, 2007). Enhancers, which determine tissue-specific gene expression, are marked by histone H3 lysine 4 mono-methylation (H3K4me1) and by the histone acetyltransferase co-factors CBP/p300 binding (Heintzman *et al.*, 2007; Visel *et al.*, 2009). Methylation of histones is carried out by histone methyltransferases (HMT) and demethylation by histone demethylases (HDM) such as members of the Jumonji protein family (Cheng and Blumenthal, 2010).

HISTONE METHYLTRANSFERASES

The Polycomb Group (PcG) proteins play an essential role during development and differentiation (Kerppola, 2009; Morey and Helin, 2010). In mammals, there are two classes of complexes, designated Polycomb Repressive Complexes 1 and 2 (PRC1 and PRC2). PRC1 is formed from 4 proteins, RING1, CBX family, PHC, and BMI1/MEL18. The core components of PRC2 are EZH2/EZH1, EED, RBAP48/46, and SUZ12 (Simon and Kingston, 2009; Morey and Helin, 2010). PRC2 regulates transcriptional repression by catalyzing the di- and tri-methylation of lysine 27 on histone H3 (H3K27me2/3). Recent genome-wide mapping studies revealed that PRC1, PRC2, and H3K27me3 occupy the same sites at the regulatory regions of several developmentally important genes, including members of the *Dlx*, *Hox*, *Pax*, *Sox*, *Gata*, and *Tbx* families (Kerppola, 2009; Simon and Kingston, 2009; Morey and Helin, 2010). The binding of PRC1 to chromatin is usually dependent on the activity of PRC2. However, PRC2 may use different recruitment mechanisms to target different downstream genes (Peng *et al.*, 2009; Shen *et al.*, 2009; Landeira *et al.*, 2010; Li *et al.*, 2010; Pasini *et al.*, 2010). PRC2 forms a stable complex with the transcriptional repressor JARID2, a member of the Jumonji C (JmjC) and ARID domain protein family (Cheng and Blumenthal, 2010). JARID2 binds to the PcG-responsive sites through the DNA-binding domain ARID (Fig. 2B) for many PRC2-regulated genes. PRC2 can also be recruited to target promoters by non-coding RNAs (Zhao *et al.*, 2008; Khalil *et al.*, 2009).

DNA METHYLATION

DNA methylation is the covalent transfer of a methyl group from S-adenosyl-L-methionine to cytosines in CpG dinucleotides (Herman and Baylin, 2003; Weber and Schübeler, 2007). Mammalian genomes are punctuated by DNA sequences containing a high number of CpG sites termed CpG islands, which

Figure 2. Epigenetic mechanisms of gene repression. **(A)** The open chromatin structure of an active gene with an unmethylated promoter region. The nucleosomes have activation marks such as acetylation (red circles) and H3K4 methylation (blue circles). The RNA Polymerase complex (Pol-II) and transcription factors (TFs) bind to the promoter and initiate transcription. **(B)** Gene repression by the Polycomb repressive complexes (PRC1 and PRC2) is mediated by the DNA-binding protein JARID2 and accompanied by H3K27 methylation (green circles), loss of H3K4 methylation, and deacetylation of nucleosomes. **(C)** DNA methylation (light blue circles) is mediated by the HP1-dependent recruitment of DNA methyltransferases (DNMTs) and the H3K9 methyltransferase G9a. Methyl-binding proteins (MeCP2 or members of the MBP family) bind to the methylated DNA and recruit histone deacetylase complexes (HDACs). Brown circles indicate H3K9 methylation. Overall, gene silencing is accompanied by the chromatin compaction, loss of histone activation marks, and removal of transcription factors. **(D)** An epigenetic mechanism dependent on microRNA, MeCP2, and Polycomb. In hepatic stellate cells, translation of the MeCP2 transcript is blocked by miR-132. Upon myofibroblast transdifferentiation, down-regulation of miR-132 enables activation of MeCP2, which binds to the PPAR γ promoter and recruits H3K9 histone methyltransferases and the HP1 repressor. In addition, MeCP2 stimulates chromatin condensation by recruiting PRC1 and PRC2. This eventually leads to repression of the PPAR γ transcription.

lack DNA methylation and associate with the majority of known gene promoters (Illingworth and Bird, 2009). In eukaryotes, the methylated CpG islands are found in non-coding regions of the genome associated with transcriptional repression (Jones and Liang, 2009), including developmental genes, repetitive sequences, and germ-line specific or imprinted genes. The changes in DNA methylation, such as hypomethylation, very often are associated with chromosome instability and activation of transposable elements in human cancers (Cheung *et al.*, 2009). DNA methylation is catalyzed by a family of closely related DNA methyltransferases (DNMT1, DNMT3a, and DNMT3b) (Hermann *et al.*, 2004). DNMT1 is a maintenance methyltransferase and is the most abundant DNA methyltransferase in mammals. It predominantly adds methylation to DNA when one strand is already methylated (hemi-methylated) (Jeltsch, 2006). The methylated DNA can recruit the methyl-CpG-binding domain proteins Kaiso, MeCP2, and members of the MBD family (Fig. 2C), which recognize 5-methylcytosines in CpG islands and participate in chromatin silencing (McCabe *et al.*, 2009).

It is important to note that DNA methylation and histone methylation are tightly controlled events in eukaryotes (Cheng and Blumenthal, 2010). The histone H3 N-terminal tail with an unmethylated lysine 4 (H3K4) is required for DNA methylation (Hu *et al.*, 2009). In addition, tri-methylation of histone H3 on lysine 9 (H3K9me3) participates in DNA methylation mediated by DNMT1 (Rottach *et al.*, 2010). The NP95 protein, which contains SET-, Ring-, and Tudor domains, is responsible for linking DNMT1 with DNA and histone H3 methylation (H3K9me3). Therefore, NP95 coordinates two major epigenetic silencing pathways, DNA methylation and histone methylation (Rottach *et al.*, 2010).

EPIGENETIC EVENTS IN INFLAMMATION

Histone Methylation and Inflammation

Jmjd3, a member of the Jumonji family, is an inducible enzyme that erases histone marks. This protein was recently found to control differentiation and cell identity in macrophages, and therefore provides a link between inflammation and reprogramming of the epigenome (Ishii *et al.*, 2009). Jmjd3 is induced in macrophages exposed to bacterial products and inflammatory cytokines, where it binds the PcG target genes and regulates their H3K27me3 levels and transcriptional activity (De Santa *et al.*, 2007). Continuous IL-4 treatment leads to activation of Jmjd3 and the release of H3K27me3 repressive marks from the STAT6 promoter. Activated STAT6 positively regulates Jmjd3 by binding to its promoter. Removal of H3K27 methylation marks by Jmjd3 triggers expression of specific inflammatory genes.

However, work by De Santa *et al.* (2009) revealed that Jmjd3 acts also through a H3K27 demethylation-independent mechanism. According to this study, Jmjd3 is preferentially recruited to transcription start sites characterized by the presence of the activation marker H3K4me3 and the presence of RNA Polymerase II complex. This study found that the binding of Jmjd3 to target genes is not accompanied by H3K27 demethylation. These findings indicate that reciprocal exchange in H3K4

and H3K27 methylation can be an important epigenetic process for the control of genes.

The mammalian genome contains several PcG target genes critical in development and differentiation (Bernstein *et al.*, 2006; Mikkelsen *et al.*, 2007). It was reported recently that some of these targets are subject to aberrant DNA methylation following chronic inflammation (Hahn *et al.*, 2008). It was shown that PcG proteins bind to the regulatory regions of target genes and recruit DNMTs (Fig. 2D) for more efficient repression. Another pathway leading to gene repression was recently described for NF- κ B/RelB-dependent silencing in severe systemic inflammation (SSI) caused by sepsis and other acute inflammatory processes (Chen *et al.*, 2009). Induction of RelB by endotoxin activation is necessary and sufficient to repress acute pro-inflammatory genes. During SSI, RelB represses these genes by inducing heterochromatin formation through direct interaction with the histone H3 lysine 9 (H3K9) methyltransferase G9a. This interaction leads to trimethylation of histone H3 on lysine 9 (H3K9me3) and subsequent recruitment of heterochromatin protein 1 (HP1). HP1 and G9a form a repressive complex at the promoters of RelB-dependent genes (Fig. 2D) and lead to the recruitment of DNMT3a/b and CpG methylation. This cooperative interaction of histone methylation and DNA methylation in response to SSI was recently reported for the TNF α promoter in blood leukocytes (El Gazzar *et al.*, 2008).

Histone Acetylation and Inflammation

Histone acetylation by HATs activates inflammatory genes, whereas increased HDAC activity results in inflammatory gene repression. In chronic obstructive pulmonary disease, airway biopsies, and alveolar macrophages, increased acetylation of histones at the promoter region of inflammatory genes is mediated by NF- κ B. The increase in histone acetylation is associated with decreased histone deacetylase (HDAC) activity. For example, promoters of several pro-inflammatory cytokines (IL-1, IL-2, IL-8, and IL-12) are rapidly acetylated by CBP/p300, leading to transcriptional activation, and display reduced HDAC activity (Villagra *et al.*, 2010). The recruitment of HDACs, in contrast, leads to histone deacetylation and gene repression. HDACs regulate transcription of both pro- and anti-inflammatory cytokines through their recruitment to gene promoters *via* corepressor complexes and transcription factors such as FOXp3, STATs, GATAs, ZEB1, and NF- κ B (Villagra *et al.*, 2010).

NF- κ B is tightly controlled by the I κ B kinase complex IKK- α in response to cytokine treatment (Ghosh and Karin, 2009). IKK- α binds to the NF- κ B-dependent promoters with the assistance of the Polymerase II complex and CBP, where it acetylates histone H3 at Lys9 (Yang *et al.*, 2009) and phosphorylates histone H3 at Ser10 (Anest *et al.*, 2003). This cytokine-induced phosphorylation is critical for the subsequent CBP-mediated acetylation of histone H3 on Lys14 (Yamamoto *et al.*, 2003). Acetylation of histone H3 at the promoters of several cytokines and chemokines after inflammation results in the increased recruitment of NF- κ B to these regions (Barnes, 2009). Glucocorticoid receptor (GR) and HDAC2 can reverse this process, thus

promoting repression of NF- κ B-dependent inflammatory genes (Barnes, 2009).

DNA Methylation and Inflammation

DNA methylation is important in the regulation of inflammatory genes. Promoter hypomethylation of the Toll-like receptor 2 (*TLR2*) gene is associated with increased pro-inflammatory response to bacterial peptidoglycan in cystic fibrosis bronchial epithelial cells (Shuto *et al.*, 2006). DNA methylation and histone acetylation regulate *TLR4* in intestinal epithelial cells (Takahashi *et al.*, 2009). DNA methylation and histone modifications play an important role in the establishment of the epigenetic landscape across the TNF α locus (Sullivan *et al.*, 2007). Environmental factors including bacterial infection were shown to contribute to the epigenetic status of the genome (Barros and Offenbacher, 2009). DNA methylation of imprinted genes plays an important role in fetal development. For example, bacterial infection induces hypermethylation in the promoter of the *Igf2* gene in mice (Bobetsis *et al.*, 2007). Expression microarray studies in mice demonstrated that maternal infection was associated with changes in placental expression of key developmental genes, including several imprinted genes (Bobetsis *et al.*, 2010). Chronic colonization of the human stomach by *Helicobacter pylori* (HP) causes inflammation within the gastric mucosa and activates multiple oncogenic pathways (Ding *et al.*, 2010). HP induces alterations in the DNA methylation pattern in gastric epithelial cells (Niwa *et al.*, 2010). Katayama *et al.* (2009) showed that HP-induced DNA methylation in the *Runx3* locus causes loss of expression in gastric epithelial cells.

MicroRNAs and Inflammation

MicroRNAs (miRNAs) are small non-coding RNAs that negatively regulate the expression of target genes at the post-transcriptional level. miRNAs are transcribed as long preliminary transcripts and, after cleavage by the Drosha and Pasha complexes in the nucleus, translocate to the cytoplasm to be processed by Dicer into 18- to 24-bp miRNA duplexes. The RNA-induced silencing complex (RISC) incorporates these short RNA duplexes and binds to the 3' untranslated region (3'UTR) of specific messenger RNAs for subsequent degradation or translational repression.

Recent investigations implicated miRNAs in the regulation of development, differentiation, and disease (Friedman *et al.*, 2009; Sonkoly and Pivarcsi, 2009). miR-146a limits Toll-like receptor signaling by blocking the signaling molecule TRAF6 (Taganov *et al.*, 2006), and miR-155 targets the lipid phosphatase SHIP1 (O'Connell *et al.*, 2009), an important signal for macrophage activation. miR-132 has anti-inflammatory effects by binding acetylcholine (ACH) mRNA, a critical inhibitor of peripheral inflammation (Shaked *et al.*, 2009). The exposure of cultured macrophages to lipopolysaccharide (LPS) leads to up-regulation of miR-155, which targets the mRNA for CCAAT/enhancer binding protein Beta (C/EBP Beta), implicated in the regulation of pro-inflammatory cytokines during macrophage

activation and the acute phase response (Worm *et al.*, 2009). Studies by Liu *et al.* (2009) demonstrated that the induction of miR-147 by TLR prevents excessive inflammatory response through a negative-feedback loop mechanism. TLR stimulation induces miR-147 and requires activation of both NF- κ B and IRF3. Furthermore, miR-147 attenuates the TLR-induced inflammatory response in macrophages (Liu *et al.*, 2009). In another study, miR-105 was shown to modulate TLR-2 translation in human gingival keratinocytes (Benakanakere *et al.*, 2009). It was recently suggested that the TLR4-dependent reprogramming of inflammatory genes is mediated by two distinct levels of regulation (El Gazzar and McCall, 2010). The first level is transcriptional control mediated by epigenetic modifications, and the second level is regulated by the TLR4-dependent differential expression of miRNAs (miR-221, miR-579, and miR-125b).

About 30% of all human genes are estimated to be potential miRNA targets (Lewis *et al.*, 2005; Xie *et al.*, 2005). Among them are genes encoding the epigenetic markers EZH2, DNMT3a and DNMT3b, and HDACs (Valeri *et al.*, 2009). miR-29 can reverse aberrant methylation in lung cancer by targeting DNMT3a and DNMT3b (Fabbri *et al.*, 2007), and miR-143 regulates DNMT3a in colorectal cancer (Ng *et al.*, 2009). miR-29 promotes osteogenesis by targeting HDAC4 (H Li *et al.*, 2009), and miR-2861 controls osteoblast differentiation by repressing HDAC5 (Z. Li *et al.*, 2009). The cartilage-specific miR-140 regulates HDAC4 (Tuddenham *et al.*, 2006), and MiR-449a targets HDAC1 in prostate cancer (Noonan *et al.*, 2009). However, the action of microRNA on epigenetic markers can be indirect. One example of this is the viral microRNA K12-4-5p, which blocks the retinoblastoma (Rb)-like protein 2 (Rb12) transcript, a known repressor of DNMT1, 3a, and 3b mRNA levels (Lu *et al.*, 2010). Expression of EZH2 is blocked by miR-101 (Varambally *et al.*, 2008; Friedman *et al.*, 2009), miR-26a (Sander *et al.*, 2008; Wong and Tellam, 2008), and miR-214 (Juan *et al.*, 2009). In the latter case, EZH2, the catalytic subunit of PRC2, and miR-214 establish a negative regulatory loop controlling PcG-dependent gene expression. PcG proteins repress transcription of miR-214 in undifferentiated skeletal muscle cells. Differentiation coincides with the PcG disengagement and activation of miR-214 transcription. miR-214, in turn, targets EZH2 (Juan *et al.*, 2009).

In summary, we considered three different epigenetic processes of gene repression: DNA methylation, histone modifications, and targeting by microRNAs. Recently, an epigenetic relay pathway was described to explain the repression of PPAR γ transcription (Mann *et al.*, 2009). This pathway (Fig. 2D) is dependent on a precisely controlled mechanism, which involves MeCP2, EZH2, and miR-132. The authors showed that down-regulation of miR-132 caused the release of the MeCP2 translational block. Consequently, MeCP2 mediates histone H3 methylation on Lys9 and Lys27 (H3K9 and H3K27) through the recruitment of HP1/G9a and PRC1/2 complexes at the PPAR γ promoter region (Mann *et al.*, 2009).

Figure 3. The regulatory circuit during oncogenic transformation. NF- κ B, IL6, let-7 microRNA, and Lin28B are key components of the positive feedback loop underlying the epigenetic switch from normal to transformed cells. The switch is induced by an initial inflammatory signal (Src activation) that activates NF- κ B, which turns on IL6 transcription and inhibits let-7 microRNA via Lin28B. The resulting high levels of IL6 activate NF- κ B, thereby completing the positive feedback loop that maintains the transformed phenotype.

INFLAMMATION AND CANCER

Inflammation contributes to the initiation and development of cancer (Rajput and Wilber, 2010). Until recently, the mechanistic aspects of this phenomenon were not clearly understood. The study by Iliopoulos *et al.* (2009) addressed this question by proposing a model that links inflammation to the oncogenic transformation. This model, based on a positive feedback loop mechanism, involves key molecular players: NF- κ B, RNA-binding protein Lin-28, let-7 microRNA, and IL-6 cytokine (Fig. 3). Activation of the Src oncoprotein triggered the inflammatory response critical for cellular transformation via a NF- κ B-dependent mechanism (Page *et al.*, 2009). NF- κ B activated transcription of Lin-28, which in turn inhibited the let-7 microRNA. let-7 repressed cellular transformation by blocking IL-6. Therefore, NF- κ B activation and subsequent repression of let-7 resulted in a dramatic increase of IL-6, which is necessary

for cellular transformation. STAT3 is a key IL-6 target that mediates oncogenic transformation.

EPIGENETICS IN HUMAN DISEASES AND AGING

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by the progressive destruction of articular cartilage and bone. Studies showed that epigenetic modifications such as genomic DNA methylation, histone acetylation, and miRNAs contribute to the pathogenesis of RA (Strietholt *et al.*, 2008; Maciejewska Rodrigues *et al.*, 2009; Brooks *et al.*, 2010). The global DNA methylation pattern in RA synovial fibroblasts was shown to be reduced compared with that of cells derived from healthy controls (Karouzakis *et al.*, 2009).

Several studies have suggested a central role for chronic inflammation in the pathogenesis of chronic obstructive pulmonary disease (COPD) and lung cancer (Bowman *et al.*, 2009; Lee *et al.*, 2009; Yao and Rahman, 2009). Recent evidence implicates epigenetic modifications in the development of tolerance in macrophages and T-cell function (Adcock *et al.*, 2007). Reduced HDAC2 expression and activity are reported in lung macrophages, biopsies, and blood cells from patients with COPD, severe asthma, and smoking asthma (Adcock *et al.*, 2007).

Age-associated changes in immune response increase the risk of infection and promote inflammation and increased reactivity to self-antigens and cancer. It has been suggested that age-associated hypomethylation of the DNA may be the cause of chronic inflammation and cancer (Agrawal *et al.*, 2010). El Mezayen *et al.* (2009) showed the age-dependent up-regulation of the *IL-23p19* gene expression associated with H3K4 methylation in dendritic cells.

Periodontitis is a multifactorial infection characterized by inflammation and destruction of tooth-supporting tissues (Gomez *et al.*, 2009). The levels of prostaglandin E and the prostaglandin-endoperoxide synthase-2 (PTGS2) increase in progressing periodontal lesions, but decrease in chronic disease. It was reported that expression of the *PTGS2* gene decreases in chronic periodontitis due to promoter hypermethylation (Zhang *et al.*, 2010).

CONCLUSIONS

Knowledge about alterations in histone modifications, DNA methylation, and microRNA regulation will provide a better understanding of the molecular basis for various chronic inflammatory diseases. Progress in studies of epigenetic alterations during inflammatory response opens opportunities for the development of efficient medications for specific targets. Among the drugs currently proposed for epigenetic therapy are histone deacetylase inhibitors and demethylating agents, which target chromatin in rapidly dividing tumor cells and restore normal cell functions (Karberg, 2009). The integration of the latest technological achievements in whole-genome microarray expression profiling and chromatin immunoprecipitation-based sequencing (ChIP-seq) methods will be instrumental in the development of epigenetic drugs with greater specificity.

ACKNOWLEDGMENTS

The author thanks William Upholt and Stormy Chamberlain for the critical reading of the manuscript. This work was supported by NIDCR grants DE18412 and DE017205 and by Connecticut Stem Cell grant 09-SCB-UCHC.

REFERENCES

- Adcock IM, Tsaprouni L, Bhavsar P, Ito K (2007). Epigenetic regulation of airway inflammation. *Curr Opin Immunol* 19:694-700.
- Agrawal A, Tay J, Yang GE, Agrawal S, Gupta S (2010). Age-associated epigenetic modifications in human DNA increase its immunogenicity. *Aging* 2:93-100.
- Anest V, Hanson JL, Cogswell PC, Steinbrecher KA, Strahl BD, Baldwin AS (2003). A nucleosomal function for I κ B kinase- α in NF- κ B-dependent gene expression. *Nature* 423:659-663.
- Barnes PJ (2009). Targeting the epigenome in the treatment of asthma and chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 6:693-696.
- Barros SP, Offenbacher S (2009). Epigenetics: connecting environment and genotype to phenotype and disease. *J Dent Res* 88:400-408.
- Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, et al. (2007). High-resolution profiling of histone methylations in the human genome. *Cell* 129:823-387.
- Benakanakere MR, Li Q, Eskan MA, Singh AV, Zhao J, Galicia JC, et al. (2009). Modulation of TLR2 protein expression by miR-105 in human oral keratinocytes. *J Biol Chem* 284:23107-23115.
- Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, et al. (2006). A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* 125:315-326.
- Bobetsis YA, Barros SP, Offenbacher S (2006). Exploring the relationship between periodontal disease and pregnancy complications. *J Am Dent Assoc* 137(Suppl):7S-13S.
- Bobetsis YA, Barros SP, Lin DM, Weidman JR, Dolinoy DC, Jirtle RL, et al. (2007). Bacterial infection promotes DNA hypermethylation. *J Dent Res* 86:169-174.
- Bobetsis YA, Barros SP, Lin DM, Arce RM, Offenbacher S (2010). Altered gene expression in murine placentas in an infection-induced intrauterine growth restriction model: a microarray analysis. *J Reprod Immunol* 85:140-148.
- Bowman RV, Wright CM, Davidson MR, Francis SM, Yang IA, Fong KM (2009). Epigenomic targets for the treatment of respiratory disease. *Expert Opin Ther Targets* 13:625-640.
- Brooks WH, Le Dantec C, Pers JO, Youinou P, Renaudineau Y (2010). Epigenetics and autoimmunity. *J Autoimmun* 34:J207-J219.
- Campos EI, Reinberg D (2009). Histones: annotating chromatin. *Annu Rev Genet* 43:559-599.
- Chen X, El Gazzar M, Yoza BK, McCall CE (2009). The NF- κ B factor RelB and histone H3 lysine methyltransferase G9a directly interact to generate epigenetic silencing in endotoxin tolerance. *J Biol Chem* 284:27857-27865.
- Cheng X, Blumenthal RM (2010). Coordinated chromatin control: structural and functional linkage of DNA and histone methylation. *Biochemistry* 49:2999-3008.
- Cheung HH, Lee TL, Rennert OM, Chan WY (2009). DNA methylation of cancer genome. *Birth Defects Res C Embryo Today* 87:335-350.
- De Santa F, Totaro MG, Prosperini E, Notarbartolo S, Testa G, Natoli G (2007). The histone H3 lysine-27 demethylase Jmjd3 links inflammation to inhibition of polycomb-mediated gene silencing. *Cell* 130:1083-1094.
- De Santa F, Narang V, Yap ZH, Tusi BK, Burgold T, Austenaa L, et al. (2009). Jmjd3 contributes to the control of gene expression in LPS-activated macrophages. *EMBO J* 28:3341-3352.
- Ding SZ, Goldberg JB, Hatakeyama M (2010). Helicobacter pylori infection, oncogenic pathways and epigenetic mechanisms in gastric carcinogenesis. *Future Oncol* 6:851-862.
- Dunning T (2009). Periodontal disease—the overlooked diabetes complication. *Nephrol Nurs J* 36:489-495.
- El Gazzar MA, McCall CE (2010). MicroRNAs distinguish translational from transcriptional silencing during endotoxin tolerance. *J Biol Chem* [Epub ahead of print, April 30, 2010] (in press).
- El Gazzar M, McCall CE (2010). MicroRNAs distinguish translational from transcriptional silencing during endotoxin tolerance. *J Biol Chem* 285:20940-20951.
- El Gazzar M, Yoza BK, Chen X, Hu J, Hawkins GA, McCall CE (2008). G9a and HP1 couple histone and DNA methylation to TNF α transcription silencing during endotoxin tolerance. *J Biol Chem* 283:32198-32208.
- El Mezayen R, El Gazzar M, Myer R, High KP (2009). Aging-dependent upregulation of IL-23p19 gene expression in dendritic cells is associated with differential transcription factor binding and histone modifications. *Aging Cell* 8:553-565.
- Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, et al. (2007). MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proc Natl Acad Sci USA* 104:15805-15810.
- Feinberg AP (2007). Phenotypic plasticity and the epigenetics of human disease. *Nature* 447:433-440.
- Friedman JM, Jones PA, Liang G (2009). The tumor suppressor microRNA-101 becomes an epigenetic player by targeting the polycomb group protein EZH2 in cancer. *Cell Cycle* 8:2313-2314.
- Fuchs J, Demidov D, Houben A, Schubert I (2006). Chromosomal histone modification patterns—from conservation to diversity. *Trends Plant Sci* 11:199-208.
- Ghosh S, Karin M (2009). Missing pieces in the NF- κ B puzzle. *Cell* 109(Suppl):81S-96S.
- Gomez RS, Dutra WO, Moreira PR (2009). Epigenetics and periodontal disease: future perspectives. *Inflamm Res* 58:625-629.
- Hahn MA, Hahn T, Lee DH, Esworthy RS, Kim BW, Riggs AD, et al. (2008). Methylation of polycomb target genes in intestinal cancer is mediated by inflammation. *Cancer Res* 68:10280-10289.
- Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW, Hawkins RD, et al. (2007). Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nat Genet* 39:311-318.
- Herman JG, Baylin SB (2003). Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med* 349:2042-2054.
- Hermann A, Gowher H, Jeltsch A (2004). Biochemistry and biology of mammalian DNA methyltransferases. *Cell Mol Life Sci* 61:2571-2587.
- Hu JL, Zhou BO, Zhang RR, Zhang KL, Zhou JQ, Xu GL (2009). The N-terminus of histone H3 is required for de novo DNA methylation in chromatin. *Proc Natl Acad Sci USA* 106:22187-22192.
- Juan AH, Kumar RM, Marx JG, Young RA, Sartorelli V (2009). Mir-214-dependent regulation of the polycomb protein Ezh2 in skeletal muscle and embryonic stem cells. *Mol Cell* 36:61-74.
- Iliopoulos D, Hirsch HA, Struhl K (2009). An epigenetic switch involving NF- κ B, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. *Cell* 139:693-706.
- Illingworth RS, Bird AP (2009). CpG islands—‘a rough guide’. *FEBS Lett* 583:1713-1720.
- Ishii M, Wen H, Corsa CA, Liu T, Coelho AL, Allen RM, et al. (2009). Epigenetic regulation of the alternatively activated macrophage phenotype. *Blood* 114:3244-3254.
- Jeltsch A (2006). Molecular enzymology of mammalian DNA methyltransferases. *Curr Top Microbiol Immunol* 301:203-225.
- Jones PA, Liang G (2009). Rethinking how DNA methylation patterns are maintained. *Nat Rev Genet* 10:805-811.
- Karberg S (2009). Switching on epigenetic therapy. *Cell* 139:1029-1031.
- Karouzakis E, Gay RE, Gay S, Neidhart M (2009). Epigenetic control in rheumatoid arthritis synovial fibroblasts. *Nat Rev Rheumatol* 5:266-272.
- Katayama Y, Takahashi M, Kuwayama H (2009). Helicobacter pylori causes runx3 gene methylation and its loss of expression in gastric epithelial cells, which is mediated by nitric oxide produced by macrophages. *Biochem Biophys Res Commun* 388:496-500.
- Kerppola TK (2009). Polycomb group complexes—many combinations, many functions. *Trends Cell Biol* 19:692-704.
- Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, et al. (2009). Many human large intergenic noncoding RNAs associate

- with chromatin-modifying complexes and affect gene expression. *Proc Natl Acad Sci USA* 106:11667-11672.
- Khansari N, Shakiba Y, Mahmoudi M (2009). Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. *Recent Pat Inflamm Allergy Drug Discov* 3:73-80.
- Lal G, Zhang N, van der Touw W, Ding Y, Ju W, Bottinger EP, *et al.* (2009). Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation. *J Immunol* 182:259-273.
- Lan F, Shi Y (2009). Epigenetic regulation: methylation of histone and non-histone proteins. *Sci China C Life Sci* 52:311-322.
- Landeira D, Sauer S, Poot R, Dvorkina M, Mazzarella L, Jørgensen HF, *et al.* (2010). Jarid2 is a PRC2 component in embryonic stem cells required for multi-lineage differentiation and recruitment of PRC1 and RNA polymerase II to developmental regulators. *Nat Cell Biol* 12:618-624.
- Lee G, Walser TC, Dubinett SM (2009). Chronic inflammation, chronic obstructive pulmonary disease, and lung cancer. *Curr Opin Pulm Med* 15:303-307.
- Lewis BP, Burge CB, Bartel DP (2005). Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 120:15-20.
- Li G, Margueron R, Ku M, Chambon P, Bernstein BE, Reinberg D (2010). Jarid2 and PRC2, partners in regulating gene expression. *Genes Dev* 15:368-380.
- Li H, Xie H, Liu W, Hu R, Huang B, Tan YF, *et al.* (2009). A novel microRNA targeting HDAC5 regulates osteoblast differentiation in mice and contributes to primary osteoporosis in humans. *J Clin Invest* 119:3666-3677.
- Li Z, Hassan MQ, Jafferji M, Aqeilan RI, Garzon R, Croce CM, *et al.* (2009). Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. *J Biol Chem* 284:15676-15684.
- Liu G, Friggeri A, Yang Y, Park YJ, Tsuruta Y, Abraham E (2009). miR-147, a microRNA that is induced upon Toll-like receptor stimulation, regulates murine macrophage inflammatory responses. *Proc Natl Acad Sci USA* 106:15819-15824.
- Lu F, Stedman W, Yousef M, Renne R, Lieberman PM (2010). Epigenetic regulation of KSHV latency by viral-encoded microRNAs that target Rta and the cellular Rb12-DNMT pathway. *J Virol* 84:2697-2706.
- Maciejewska Rodrigues H, Jüngel A, Gay RE, Gay S (2009). Innate immunity, epigenetics and autoimmunity in rheumatoid arthritis. *Mol Immunol* 47:12-18.
- Mann J, Chu DC, Maxwell A, Oakley F, Zhu NL, Tsukamoto H, *et al.* (2009). MeCP2 controls an epigenetic pathway that promotes myofibroblast transdifferentiation and fibrosis. *Gastroenterology* 138:705-714.
- McCabe MT, Brandes JC, Vertino PM (2009). Cancer DNA methylation: molecular mechanisms and clinical implications. *Clin Cancer Res* 15:3927-3937.
- Medzhitov R (2008). Origin and physiological roles of inflammation. *Nature* 454:428-435.
- Medzhitov R, Horng T (2009). Transcriptional control of the inflammatory response. *Nat Rev Immunol* 9:692-703.
- Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, *et al.* (2007). Genome-wide maps of chromatin state in pluripotent and differentiated cells. *Nature* 448:553-560.
- Morey L, Helin K (2010). Polycomb group protein-mediated repression of transcription. *Trends Biochem Sci* 35:323-332.
- Ng EK, Tsang WP, Ng SS, Jin HC, Yu J, Li JJ, *et al.* (2009). MicroRNA-143 targets DNA methyltransferases 3A in colorectal cancer. *Br J Cancer* 101:699-706.
- Niwa T, Tsukamoto T, Toyoda T, Mori A, Tanaka H, Maekita T, *et al.* (2010). Inflammatory processes triggered by *Helicobacter pylori* infection cause aberrant DNA methylation in gastric epithelial cells. *Cancer Res* 70:1430-1440.
- Noonan EJ, Place RF, Pookot D, Basak S, Whitson JM, Hirata H, *et al.* (2009). miR-449a targets HDAC-1 and induces growth arrest in prostate cancer. *Oncogene* 28:1714-1724.
- O'Connell RM, Chaudhuri AA, Rao DS, Baltimore D (2009). Inositol phosphatase SHIP1 is a primary target of miR-155. *Proc Natl Acad Sci USA* 106:7113-7118.
- Page TH, Smolinska M, Gillespie J, Urbaniak AM, Foxwell BM (2009). Tyrosine kinases and inflammatory signalling. *Curr Mol Med* 9:69-85.
- Pasini D, Cloos PA, Walfridsson J, Olsson L, Bukowski JP, Johansen JV, *et al.* (2010). JARID2 regulates binding of the Polycomb repressive complex 2 to target genes in ES cells. *Nature* 464:306-310.
- Peng JC, Valouev A, Swigut T, Zhang J, Zhao Y, Sidow A, *et al.* (2009). Jarid2/Jumonji coordinates control of PRC2 enzymatic activity and target gene occupancy in pluripotent cells. *Cell* 139:1290-1302.
- Rajput S, Wilber A (2010). Roles of inflammation in cancer initiation, progression, and metastasis. *Front Biosci* 2:176-183.
- Rottach A, Frauer C, Pichler G, Bonapace IM, Spada F, Leonhardt H (2010). The multi-domain protein Np95 connects DNA methylation and histone modification. *Nucleic Acids Res* 38:1796-1804.
- Safronova O, Morita I (2010). Transcriptome remodeling in hypoxic inflammation. *J Dent Res* 89:430-444 [review].
- Sander S, Bullinger L, Klapproth K, Fiedler K, Kestler HA, Barth TF, *et al.* (2008). MYC stimulates EZH2 expression by repression of its negative regulator miR-26a. *Blood* 112:4202-4212.
- Shaked I, Meerson A, Wolf Y, Avni R, Greenberg D, Gilboa-Geffen A, *et al.* (2009). MicroRNA-132 potentiates cholinergic anti-inflammatory signaling by targeting acetylcholinesterase. *Immunity* 31:965-973.
- Shen X, Kim W, Fujiwara Y, Simon MD, Liu Y, Mysliwiec MR, *et al.* (2009). Jumoni modulates polycomb activity and self-renewal versus differentiation of stem cells. *Cell* 139:1303-1314.
- Shuto T, Furuta T, Oba M, Xu H, Li JD, Cheung J, *et al.* (2006). Promoter hypomethylation of Toll-like receptor-2 gene is associated with increased proinflammatory response toward bacterial peptidoglycan in cystic fibrosis bronchial epithelial cells. *FASEB J* 20:782-784.
- Simon JA, Kingston RE (2009). Mechanisms of polycomb gene silencing: knowns and unknowns. *Nat Rev Mol Cell Biol* 10:697-708.
- Sonkoly E, Pivarcsi A (2009). microRNAs in inflammation. *Int Rev Immunol* 28:535-561.
- Strietholt S, Maurer B, Peters MA, Pap T, Gay S (2008). Epigenetic modifications in rheumatoid arthritis. *Arthritis Res Ther* 10:219.
- Sullivan KE, Reddy AB, Dietzmann K, Suriano AR, Kocieda VP, Stewart M, *et al.* (2007). Epigenetic regulation of tumor necrosis factor alpha. *Mol Cell Biol* 27:5147-5160.
- Taganov KD, Boldin MP, Chang KJ, Baltimore D (2006). NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci USA* 103:12481-12486.
- Takahashi K, Sugi Y, Hosono A, Kaminogawa S (2009). Epigenetic regulation of TLR4 gene expression in intestinal epithelial cells for the maintenance of intestinal homeostasis. *J Immunol* 183:6522-6529.
- Thompson RF, Einstein FH (2010). Epigenetic basis for fetal origins of age-related disease. *J Women's Health* 19:581-587.
- Timmermann S, Lehmann H, Poleskaya A, Harel-Bellan A (2001). Histone acetylation and disease. *Cell Mol Life Sci* 58:728-736.
- Tuddenham L, Wheeler G, Ntounia-Fousara S, Waters J, Hajihosseini MK, Clark I, *et al.* (2006). The cartilage specific microRNA-140 targets histone deacetylase 4 in mouse cells. *FEBS Lett* 580:4214-4217.
- Valeri N, Vannini I, Fanini F, Calore F, Adair B, Fabbri M (2009). Epigenetics, miRNAs, and human cancer: a new chapter in human gene regulation. *Mamm Genome* 20:573-580.
- Varambally S, Cao Q, Mani RS, Shankar S, Wang X, Ateeq B, *et al.* (2008). Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. *Science* 322:1695-1699.
- Villagra A, Sotomayor EM, Seto E (2010). Histone deacetylases and the immunological network: implications in cancer and inflammation. *Oncogene* 29:157-173.
- Visel A, Blow MJ, Li Z, Zhang T, Akiyama JA, Holt A, *et al.* (2009). ChIP-seq accurately predicts tissue-specific activity of enhancers. *Nature* 457:854-858.
- Weber M, Schübeler D (2007). Genomic patterns of DNA methylation: targets and function of an epigenetic mark. *Curr Opin Cell Biol* 19:273-280.
- Wells AD (2009). New insights into the molecular basis of T cell anergy: anergy factors, avoidance sensors, and epigenetic imprinting. *J Immunol* 182:7331-7341.
- Wierda RJ, Geutskens SB, Jukema JW, Quax PH, van den Elsen PJ (2010). Epigenetics in atherosclerosis and inflammation. *J Cell Mol Med* [Epub ahead of print, January 30, 2010] (in press).

- Wong CF, Tellam RL (2008). MicroRNA-26a targets the histone methyltransferase enhancer of zeste homolog 2 during myogenesis. *J Biol Chem* 283:9836-9843.
- Worm J, Stenvang J, Petri A, Frederiksen KS, Obad S, Elmén J, *et al.* (2009). Silencing of microRNA-155 in mice during acute inflammatory response leads to derepression of c/ebp beta and down-regulation of G-CSF. *Nucleic Acids Res* 37:5784-5792.
- Xie X, Lu J, Kulbokas EJ, Golub TR, Mootha V, Lindblad-Toh K, *et al.* (2005). Systematic discovery of regulatory motifs in human promoters and 3' UTRs by comparison of several mammals. *Nature* 434:338-345.
- Yamamoto Y, Verma UN, Prajapati S, Kwak YT, Gaynor RB (2003). Histone H3 phosphorylation by IKK-alpha is critical for cytokine-induced gene expression. *Nature* 423:655-659.
- Yang J, Park Y, Zhang H, Xu X, Laine GA, Dellsperger KC, *et al.* (2009). Feed-forward signaling of TNF-alpha and NF-kappaB via IKK-beta pathway contributes to insulin resistance and coronary arteriolar dysfunction in type 2 diabetic mice. *Am J Physiol Heart Circ Physiol* 296: H1850-H1858.
- Yao H, Rahman I (2009). Current concepts on the role of inflammation in COPD and lung cancer. *Curr Opin Pharmacol* 9:375-383.
- Zhang S, Barros SP, Niculescu MD, Moretti AJ, Preisser JS, Offenbacher S (2010). Alteration of PTGS2 promoter methylation in chronic periodontitis. *J Dent Res* 89:133-137.
- Zhao J, Sun BK, Erwin JA, Song JJ, Lee JT (2008). Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. *Science* 322:750-756.