Epigenetic Regulation of Gene Expression in the Inflammatory Response and Relevance to Common Diseases

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Epigenetics can be defined as all the meiotically and mitotically inherited changes in gene expression that are not encoded in the DNA sequence itself. Epigenetic modifications of chromatin and DNA have been recognized as important permissive and suppressive factors in controlling the expressed genome via gene transcription. Two major epigenetic mechanisms are the posttranslational modification of histone proteins in chromatin and the methylation of DNA itself, which are regulated by distinct, but coupled, pathways. It is clear that the epigenetic state is a central regulator of cellular development and activation. Emerging evidence suggests a key role for epigenetics in human pathologies, including in inflammatory and neoplastic disorders. The epigenome is influenced by environmental factors throughout life. Nutritional factors can have profound effects on the expression of specific genes by epigenetic modification, and these may be passed on to subsequent generations with potentially detrimental effects. Many cancers are associated with altered epigenetic profiles, leading to altered expression of the genes involved in cell growth or differentiation. Autoimmune and neoplastic diseases increase in frequency with increasing age, with epigenetic dysregulation proposed as a potential explanation. In support of this hypothesis, studies in monozygotic twins revealed increasing epigenetic differences with age. Differences in methylation status of CpG sites, monoallelic silencing, and other epigenetic regulatory mechanisms have been observed in key inflammatory response genes. The importance of the epigenome in the pathogenesis of common human diseases is likely to be as significant as that of traditional genetic mutations. With advances in technology, our understanding of this area of biology is likely to increase rapidly in the near future. *J Periodontol 2008;79:1514-1519.*

KEY WORDS

DNA methylation; epigenetics; gene expression; histones; inflammatory disease; pathogenesis.

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Within a population, there exist large differences in clinical responses to inflammatory stimuli, suggesting that any gene important to the inflammatory response is likely subject to variability within that population. Genetic polymorphisms in a large number of genes have been shown to quantitatively and qualitatively affect the immune and inflammatory responses, with effects on susceptibility or severity of a large number of diseases with an inflammatory component. Our understanding of the cellular and molecular mechanisms governing the inflammatory response in human diseases has progressed rapidly. Although the study of genetics provided the first clues in unraveling the factors that cause or contribute to the differences in inflammatory responses between individuals, there are many differences whose mechanisms are not genetic in origin. Recently, there has been a greater appreciation of the role of epigenetics, meiotically and mitotically heritable changes in gene expression that are not coded in the DNA sequence itself,[1] in the pathogenesis of common disease, especially in those involving the inflammatory response.[2,3] This article briefly discusses the epigenetic mechanisms of gene regulation, environmental, dietary, and aging effects on the epigenetic signature of the genome, and the evidence of a role for

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epigenetics in common diseases and inflammation. Finally, we will highlight emerging evidence indicating that such epigenetic factors may be important in understanding the origins in variations in the inflammatory response between individuals.

EPIGENETIC MECHANISMS

Epigenetic mechanisms result in heritable modifications in the expression of genes that are independent from DNA coding variability. These phenomena have been recognized as important permissive and suppressive factors in controlling the expressed genome via gene transcription. Two major epigenetic mechanisms are the posttranslational modification of histone proteins in chromatin and the methylation of DNA itself. These are regulated by distinct, but coupled, pathways.

DNA methylation

Within the nucleus, chromosomal DNA is tightly associated with proteins, and these interactions form the ordered structure known as chromatin. DNA itself can be modified, via covalent addition of methyl groups, catalyzed by enzymes known as DNA methyltransferases. This primarily happens at specific dinucleotide sites along the genome, i.e., cytosines 5′ of guanines, or at CpG sites. In fact, 40% of genes contain CpG-rich islands upstream from their transcriptional start site, and up to 70% to 80% of all CpG dinucleotides in the genome are methylated.[4] When such widespread methylation occurs in these regulatory regions, it likely silences the gene by interfering with the access of transcription factors to the promoter region.[5] This high degree of methylation across the genome confers long-term epigenetic silencing and is especially important for particular sequences known as transposons. Transposons are DNA sequences that can move around to different positions within the genome, and their movement can cause mutations or alter gene expression levels. One group of transposons, which is composed of sequences of retroviral origin, retroviruses, accounts for up to 8% of the human genome and, in some cases, is directly transcriptionally regulated by CpG methylation.[6] Global demethylation of transposable elements may contribute to pathways that ultimately result in the initiation and progression of cancer and other diseases. As one would expect, there is an inverse correlation between DNA methylation and gene expression.[4]

Histone modification

The individual units that make up chromatin are referred to as nucleosomes, which consist of 147 base pairs of DNA and an octamer of associated histone proteins (two each of H2A, H2B, H3, and H4).[7] Gene expression is controlled, in part, by these protein complexes that continuously pack and unpack the chromosomal DNA from inaccessible condensed nucleosomal particles to accessible relaxed nucleosomal particles.[8] Specifically, various covalent posttranslational modifications of histone proteins regulate this process, ultimately regulating gene expression. Normally, histone proteins are positively charged and form tight electrostatic associations with negatively charged DNA, which leads to chromatin compaction and impaired gene expression. However, if the positive charges of the histone proteins are changed by the addition of an acetyl group (i.e., histone acetylation), the DNA and histones form a more relaxed (open) configuration, which favors gene expression. The addition and removal of these acetyl groups is performed by specific enzymes known as histone acetyltransferases and histone deacetylases (HDAC), respectively. Other posttranslational histone modifications include methylation and phosphorylation; depending on its location, histone methylation may be a marker of active or inactive regions of chromatin.[9]

It was established that the processes of DNA methylation and histone modification are linked. For example, when the CpG island of a promoter becomes methylated, methyl-CpG binding proteins bind to these methylated CpGs and recruit histone deacetylases. Deacetylation of histone proteins then occurs, resulting in strong electrostatic interaction between the positively charged acetylated histone residues and the negatively charged DNA, creating a condensed nucleosome particle and repressing gene expression.[8]

GENE EXPRESSION REGULATION

Although cells with such diverse functions as hepatocytes, neurons, and B cells possess the same genomic DNA, they clearly have significantly different gene expression patterns. It is becoming clear that the epigenetic signature, the pattern of unique histone modifications and DNA methylation at gene loci, is a central regulator of distinct cell speciation. One of the best-described examples is found at the insulin gene.[10] The insulin gene in insulin-producing pancreatic islet cells displays histone modifications typical of active genes (e.g., H4 hyperacetylation and dimethylation of H3 lysine 4). These modifications are not present in a human epithelial cell line (HeLa) and in human bone marrow–derived mesenchymal stem cells, which instead have elevated levels of H3 lysine 9 dimethylation that are typical of inactive genes. Genome-wide chromatin-state maps were recently generated for mouse embryonic stem cells, neural progenitor cells, and embryonic fibroblasts, which show specific methylation patterns associated with the gene expression state across the genome.[11] For

example, H3 lysine 4 and lysine 27 trimethylation effectively discriminates genes that are expressed, poised for expression, or stably repressed and, therefore, reflects cell state and lineage potential.

Normally, it is presumed that both copies of each gene inherited from both parents are expressed simultaneously at equivalent levels. However, there are three notable situations in which only one of the alleles is expressed; each is an example of epigenetic gene regulation. First, and probably the most dramatic example, is X-chromosome inactivation, a process by which one of the two copies of the X-chromosome present in females is inactivated. The inactivated X-chromosome possesses high levels of DNA methylation, low levels of histone acetylation, low levels of histone H3 lysine 4 methylation, and high levels of histone H3 lysine 9 methylation, all of which are associated with gene silencing.[12]

Second, genomic imprinting, or allelic silencing, is a phenomenon by which certain individual genes are expressed in a parent-of-origin–specific manner throughout a tissue or organism. These imprinted genes are expressed only from the allele inherited from the mother or the father. This phenomenon has been observed at ~80 different genes, located in clusters, with most having roles in cell development and proliferation or behavior.[13] For example, the gene encoding insulin-like growth factor-2 is only expressed from the allele inherited from the father.[14] As well, some heritable diseases have been known to involve genetic imprinting: one of which is Prader-Willi syndrome, which features muscle weakness, obesity, learning difficulties, and infertility. This disease is due to a lack of expression of imprinted genes within chromosome 15q11-q13 and is caused by a deletion or epigenetic silencing.[15]

Third, there is evidence of epigenetic regulation of autosomal gene expression, providing a mechanism by which individual cells exist as a mosaic with respect to gene expression patterns within tissue. For example, in a recent genome-wide investigation[16] of allele-specific transcription of ~4,000 genes, >300 were subject to random monoallelic expression in cells, i.e., only the maternal or paternal allele was randomly expressed. Of these, a significant majority (>80%) also displayed biallellic expression (expression of both alleles) in individual cells. Presumably, such allele-specific choices in individual cells, taken together with clonal expansion to form tissues, may lead to macroscopic patches of tissue within an organ with subtly different properties.[16] Clearly, epigenetic regulation, from the whole organism to the individual cell, is a powerful form of gene expression and generates diversity at the cellular level. The influence of external forces on these various mechanisms of epigenetic regulation creates another factor contributing to the variability in gene expression.

EPIGENETICS: EFFECTS OF DIET AND AGING

The epigenome is influenced by environmental factors throughout life. Nutritional factors can have profound effects on the expression of specific genes by epigenetic modification; these may be passed on to subsequent generations, with potentially detrimental effects. For example, it is well known that folate deficiency is associated with open neural tube defects.[17] Because folate is an essential factor in the conversion of methionine to S-adenosylmethionine, the main methyl group donor in DNA methylation reactions, dietary deficiency in folate leads to genomic hypomethylation. Other dietary components, such as selenium, arsenic, and polyphenols, may also influence the state of DNA methylation with potential consequences for diseases such as cancer.[18] However, the state of hypomethylation is reversible, because folate therapy was shown to restore DNA methylation to normal levels, correcting the patterns of gene expression administration.[19] The resulting folic acid fortification in foods has resulted in significant reductions in the incidence of open neural tube defects in newborns.[20]

The role of epigenetics in aging is an emerging field of research. It is now known that aged organisms, i.e., those advanced in years, have modified epigenetic signatures. Typically, one observes a decrease in global CpG methylation, coupled with specific regions of hypermethylation, usually in promoter regions.[21] It is hypothesized that such epigenetic changes would result in an altered gene expression profile. Therefore, active investigations continue into potential functional relationships between these epigenetic changes and the disease pathology of common late-onset diseases.[21] Age-related epigenetic differences in humans have been best illustrated through the examination of the epigenome of sets of monozygotic twins.[22] In an examination of the global and gene-specific differences in DNA methylation and histone acetylation of 50 monozygotic twins, it was found that although twins were epigenetically very similar in early life, older twins exhibited significant differences in DNA methylation. The fact that these differences were more pronounced in twins who had different lifestyles and had spent less of their lives together further highlights the role that environmental factors have in determining the epigenetic signature.[21]

Clearly, the epigenetic signature is a dynamic entity (Fig. 1). Considering the number of elements involved and their various sites of modification, it should be no surprise that mounting evidence suggests that a disruption of the delicate balance in these epigenetic networks by environmental stimuli is a factor in a range of diseases, including cancer, inflammatory disorders, and autoimmunity.[1]

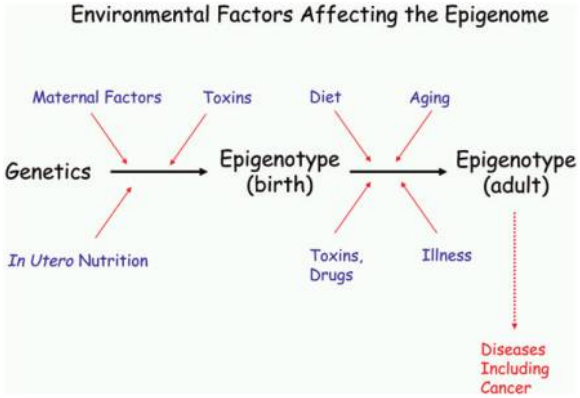

Figure 1.
The epigenetic signature is dynamic. Unlike the genetic sequence, the epigenetic signature is dynamic and changes in response to various environmental stimuli. Potential influences include toxin exposure, smoking, illness, drugs, diet, age, in utero *nutrition, and family history, each of which may affect the epigenetic signature to varying degrees at different key points in development. These epigenetic modifications may be passed on to subsequent generations with potentially detrimental effects.*

EPIGENETICS IN COMMON DISEASES

Although it is clear that the epigenetic state is a central regulator of cellular development and activation, emerging evidence implicates epigenetics in the pathogenesis of common diseases. For example, it was recognized that abnormal patterns of DNA methylation occurred in cancer cells.[18] Specifically, a global state of hypomethylation is observed, along with a seemingly contradictory state of hypermethylation of many gene promoters, a pattern similarly observed in aging. Although a mechanistic link between promoter hypermethylation and the aging process does not exist, per se, it is possible to associate the accumulation of methylation at the promoters of tumor suppressor and other genes during aging with the predisposition to develop cancer.[21] For example, increased promoter methylation during aging observed in the gene encoding the adhesion molecule E-cadherin is also found in malignancies of numerous tissues, including bladder cancer.[23] One can imagine how the loss of expression of an adhesion molecule may be important for cancer metastases. Other abnormal patterns of methylation are observed in smokers. Here, the demethylation of the oncogene synuclein-γ in lung tissues is evident and occurs via the downregulation of a specific DNA methyltransferase.[24] Recent evidence implicates microRNAs (miRNAs), small, non-coding RNAs that regulate the expression of many genes, in carcinogenesis via the alteration of DNA methylation. The miRNA miR-29s targets several DNA methyltransferase enzymes in lung cancer tissue, resulting in aberrant DNA methylation.[25] Thus, the low expression of DNA methyltransferases may, in part, explain the state of global hypomethylation in human cancers, providing evidence on how epigenetic mechanisms may contribute to the cancer pathogenesis.

Autoimmune and inflammatory diseases increase in frequency with increasing age, with epigenetic dysregulation proposed as a potential explanation. For example, tumor necrosis factor (TNF) is a potent cytokine with a wide range of proinflammatory activities. It is known that TNF transcription, mRNA levels, and protein secretion dramatically increase in response to endotoxin lipopolysaccharide (LPS) stimulation of macrophages. However, interindividual variation in TNF production can be significant, with up to a 40-fold difference being observed.[26] Part of this variation can be explained by genetics, i.e., polymorphisms in the TNF promoter region.[27] For example, carriage of the TNF-308A allele was associated with more severe outcomes in various infectious diseases; for example, homozygosity carries a seven-fold increased risk for death from cerebral malaria.[28]

To the best of the author's knowledge, the potential role of epigenetic variability underlying the variance in TNF production in the human population has not been studied. In a preliminary study, we examined the effect of the global demethylating agent, 5-Aza-2′ deoxycytidine (5-Aza-dC), on TNF mRNA production using the HeLa cell line. This led to a dose-dependent increase in LPS-induced stimulation of TNR mRNA expression; however, because 5-Aza-dC incorporates itself into DNA non-specifically, the alteration in TNF expression may be due to effects on TNF-regulating genes. In another set of experiments, the methylation status of the TNF promoter (–310 to +30) was examined in LPS-stimulated macrophages drawn from healthy individuals. This region of the TNF promoter contains 12 CpG dinucleotides. Lower methylation at two specific CpG sites (–304 and –245) was correlated with high production of TNF mRNA, suggesting that the methylation state of the TNF promoter may be an important factor in driving the level of TNF gene expression, and it may help to explain the origins in variation in the inflammatory response between individuals.

A number of features of autoimmune disease have been proposed to be due to epigenetic effects, including the disease discordance in monozygotic twins, the late onset of disease (most commonly in decades four and five), the parent-of-origin genetic effects, and female predominance.[29] Although significant discordance rates in monozygotic twins can be found in those with type 1 diabetes, schizophrenia, and breast cancer, the discordance rate in rheumatoid arthritis (RA) can be as high as 70% and as high as 93% for dizygotic twins.[30] RA is the most common autoimmune inflammatory joint disease, with a prevalence of 1%.

Studies are beginning to demonstrate an epigenetic component to the disease pathology. The effective treatment of RA using inhibitors of TNF and interleukin (IL)-6 confirmed that this disease is driven by an imbalance in cytokine production, featuring an excess of proinflammatory cytokines.[31] In a study[32] of the methylation status of the IL-6 promoter (–1,200 to +30) in subjects with RA compared to healthy controls, a single CpG site at –1,181 was significantly less methylated in subjects with RA, and the methylation of this dinucleotide resulted in reduced affinity with a nuclear protein in an in vitro assay.

EPIGENETICS IN INFLAMMATION

The importance of epigenetic regulatory mechanisms in controlling the immune and inflammatory responses is emerging. For example, in T cells, a small region of the promoter of the IL-2 gene demethylates shortly after activation, leading to IL-2 production.[33] The differentiation of naive CD4 T cells to the T helper 2 phenotype is characterized by the rapid acquisition of H3 acetylation at the IL-4/-13 gene clusters.[34] Modification was revealed in an elegant set of experiments[35] examining the gene-specific control of LPS-induced tolerance by chromatin. Briefly, although macrophages respond to LPS stimulation, they become hyporesponsive upon subsequent LPS stimulation. It seems that two distinct patterns of chromatic modifications occur during this hyporesponsive state: although one group of genes responsible for inflammatory molecule production (e.g., TNF and IL-6) is transiently silenced (i.e., tolerized genes), a second group of genes, which includes various antimicrobial effectors, remains primed for activation (i.e., non-tolerized). These states of tolerance are regulated by various modifications in histones associated with these two functional groups. The resulting outcome is that tolerization leads to the prevention of additional pathology associated with excessive inflammation, whereas non-tolerized genes are still able to protect the host from infection through antimicrobial means. Such dynamic epigenetic regulation is key to controlling the host inflammatory response.

CONCLUSIONS

Epigenetics is central in tissue-specific gene expression. Clearly, the epigenetic signature is dynamic and modified by environmental factors and the aging process. There is emerging evidence to suggest that such epigenetic factors may be important in understanding the origins of interindividual variations in the inflammatory response. The biologic significance of age-related epigenetic changes in the pathogenesis of common late-onset diseases, such as cancer and autoimmunity, are not clear. Yet, there have been very promising recent demonstrations of the therapeutic potential of epigenetic manipulation, especially for the use of HDAC inhibitors. Notable examples of HDAC inhibitor successes include preventing fibrosis associated with systemic sclerosis,[36] correcting defective apoptosis in RA,[37] and the suppression of inflammatory cytokine production (TNF and IL-1 and -8) and cartilage and bone destruction in a rat model of rheumatoid arthritis.[38] Such therapeutic uses may prove to be beneficial in a wide range of diseases, as characterized by the use of valproic acid, used for many years as an anticonvulsant in epilepsy and a mood stabilizer in bipolar disorder, which possesses significant HDAC inhibitor activity.[39] Emerging evidence suggests that the epigenome may be important in the pathogenesis of a large number of illnesses and is likely to be as significant as that of genetic variation; with advances in technology, our understanding of this area of biology is likely to increase rapidly in the near future.

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