An Epigenetic Clue to Diabetic Vascular Disease

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The cardiovascular complications associated with diabetes and the closely related metabolic syndrome are of increasing clinical importance throughout the world and especially in the Western hemisphere. Although insulin resistance and hyperglycemia are thought to play a pathogenic role during the progression of atherosclerosis, the precise mechanisms involved are not completely clear. One factor that has been implicated is enhanced inflammatory signaling leading to the recruitment of monocytes/macrophages to the vessel wall. Evidence suggests that high glucose (HG)-mediated increases in oxidative stress and the formation of AGEs (advanced glycation end products) result in the upregulation of cytokines, cell adhesion molecules, and matrix modifying genes that facilitate lymphocyte activation and invasion. Many of these genes are regulated by nuclear factor (NF)-κB, which has been shown to be activated under diabetic conditions in several cell types. Interestingly, in many instances, cells and animals that have been exposed to HG have been shown to maintain a diabetic cardiovascular phenotype even after lengthy exposure to normal glucose levels, resulting in the concept of “metabolic memory.” In this issue of Circulation Research, Reddy et al have examined this concept more closely in smooth muscle cells (SMCs) isolated from diabetic mice and SMCs subjected to HG. Their data suggest that the increase in cytokine expression observed in these cells may be attributable to epigenetic alterations in histone methylation that enhance the activation of these genes.

Epigenetic Regulation of Gene Expression by Histone Methylation

Genomic DNA is organized into a basic structure called a nucleosome that consists of an octamer of histone proteins (dimers of H2A, H2B, H3, and H4) wrapped approximately twice by 146 bp of genomic DNA. Although condensation of DNA into higher order chromatin is obviously required for normal cell function, it is inherently inhibitory to gene expression and gene replication and must be altered during these processes. Recent studies have shown that this is achieved by posttranslational modification of histone tails by acetylation, methylation, ubiquitylation, and phosphorylation (reviewed previously). Histone acetyl transferases such as p300 and CREB-binding protein have been shown to modify a variety of lysine residues on H3 and H4. The addition of negatively charged acetyl groups is thought to interfere with histone–DNA interactions effectively “loosening” chromatin structure and allowing increased transcription factor access to DNA. The histone deacetylases remove acetyl groups and, in general, inhibit transcription factor access and gene transcription. H3 and H4 lysines (and some arginines) can also be methylated by several families of histone methyltransferases to the mono-, di-, or trimethylated form (reviewed previously). Unlike histone acetylation, histone methylation can be associated with both gene activation and inactivation, and the effects of methylation are thought to be mediated by recruitment of additional positive or negative transacting factors. One of the most common chromatin modifications at transcriptionally active genes is methylation of histone 3 at lysine 4 (H3K4), whereas gene inactivation and heterochromatinization is strongly associated with methylation of H3K9.

Because no specific histone demethylases had been described, it was originally thought that histone methylation was a fairly permanent modification that resulted in relatively long-term changes in gene expression. However, recent studies have identified histone demethylases that catalyze the removal of methyl groups from histone lysines (reviewed previously). These findings were extremely important because they demonstrated that histone methylation, like histone acetylation, was reversible, specifically regulated by a group of enzymes, and likely to play a dynamic role in the regulation of gene expression. Lysine-specific histone demethylase 1 (LSD1) was the first histone lysine demethylase to be described and was originally isolated as a component of a multiprotein repressor complex. Subsequent studies demonstrated that this flavin-dependent amine oxidase specifically demethylated the mono- and dimethylated forms of H3K4 and that this mechanism contributed to the inhibition of neuronal gene expression in HeLa cells. A second larger class of iron-based histone demethylases characterized by the presence of the conserved Jumonji C domain has since been identified, and it is becoming clear that each histone demethylase targets specific histone lysines (ie, H3K4 versus H3K9) and specific lysine modifications (ie, the mono- versus di- versus trimethylated form) (reviewed elsewhere).

A Role for LSD1 in Diabetes-Induced Inflammatory Gene Expression in SMCs

Based on previous studies demonstrating that SMCs isolated from diabetic mice or SMCs exposed to HG exhibit proatherotic responses (ie, increased proliferation, migration, and inflammatory gene expression), Reddy et al set out to test the hypothesis that the diabetic SMC phenotype was...
facilitated by alterations in chromatin structure that led to increased inflammatory gene expression. They initially demonstrated that SMCs isolated from diabetic mice lacking the long form of the leptin receptor (db/db) exhibited increased expression of monocyte chemotactic protein (MCP)-1 and interleukin (IL)-6. Chromatin immunoprecipitation (ChIP) assays were used to show that these changes in chemokine expression were associated with increased H3K4 methylation near the NF-κB response elements within the MCP-1 and IL-6 promoters. Importantly, expression of the H3K4-specific demethylase, LSD1, was slightly reduced in these cells, and additional ChIP assays demonstrated a dramatic decrease in LSD1 recruitment to the MCP-1 and IL-6 promoters. Similar changes were seen in human vascular SMCs exposed to HG, suggesting a common mechanism for the gene expression changes observed in these models. To address whether LSD1 was directly involved in this response, small interfering RNA techniques were used to knockdown LSD1 in normal SMCs. Even a relatively modest 50% reduction of LSD1 resulted in enhanced tumor necrosis factor-α–induced MCP-1 and IL-6 expression. Finally, to assess the physiological importance of LSD1, fluorescently labeled monocyte THP-1 cells were added to monolayers of Wt and LSD1 knockdown human SMCs. As expected, knockdown of LSD1 enhanced basal and tumor necrosis factor-α–stimulated monocyte adherence. Taken together, these results suggest that LSD1 regulates inflammatory gene expression in SMCs and may contribute to metabolic memory through long-term changes in gene expression via alterations in chromatin structure.

**Remaining Questions and Future Challenges**

Although the characterization of histone posttranslational modifications has greatly enhanced our understanding of epigenetic gene regulation, many questions remain in regard to the specificity of these regulatory mechanisms. For example, very little is known about how the histone modifying enzymes are recruited to the promoters on which they act or the overall gene programs that are affected by particular enzymes or histone modifications. In addition, it is currently unclear how histone modifications converge with many of the previously established cell type–specific transcriptional regulatory mechanisms. LSD1 has already been shown to regulate the expression of a variety of other genes\(^{15,20–23}\) and higher-throughput methods will likely be required to identify the full spectrum of genes that are LSD1-dependent.\(^{24,25}\) The report by Reddy et al suggested that diabetic conditions did not affect overall H3K4 dimethylation or the expression of a control gene (cyclphilin A), suggesting at least some specificity to this response. Interestingly, the ability of LSD1 to demethylate histones within a nucleosomal context requires its interaction with the corepressor CO-REST.\(^{26}\) However, there is currently no evidence to suggest that CO-REST can mediate the recruitment of LSD1 to specific promoters. The fact that NF-κB recruitment to the MCP-1 and IL-6 promoters was also increased in db/db SMCs merits a closer examination of the effects of LSD1 on other NF-κB–dependent genes. Another difficult, but important, question is whether chromatin modifiers are recruited by specific transcription factors or whether chromatin modification is a prerequisite for transcription factor binding. Additional ChIP experiments examining the precise timing of NF-κB and LSD1 recruitment could shed light on this issue.

In summary, it is clear that gene expression patterns are dynamically regulated by alterations in chromatin structure that modulate transcription factor access to DNA. Studies on the newly described histone demethylases are beginning to characterize the specific gene programs that are regulated by this mechanism. A major future challenge to the cardiovascular field will be to determine the contributions of these (and other) chromatin modifying enzymes to cardiovascular development and the progression of cardiovascular disease.

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