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Current Epigenetic Perspective on Diabetes: Who Regulates the Regulators?

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Abstract

Intensified interest in the development of pharmacological compounds that manipulate gene control highlights the importance of understanding the key players and molecular events driving gene function. Epigenetic research too frequently focuses on a single chromatinized modification, often at a limited number of loci, and without context for other determinants of transcription. This perception is problematic because it implies an oversimplification of the vastly complex and multidimensional network of gene control. It overlooks the interactions of chromatin modifying enzymes and transcription factors, and seldom addresses the molecular events and signaling cues that influence the executive enzymatic machinery that regulate the epigenome. Here we discuss the connectivity and complexity of epigenetic regulation in the context of chromatin modifications and transcription factors. Using the example of the Set7 methyltransferase, we describe recent observations that expand the understanding of chromatin biology.

Keywords: Epigenetic; Gene; Epigenome; Set7/9; Diabetes; Vascular complications; Metabolic memory; Chromatin; Transcription

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Dynamic Chromatin

Site-selective DNA-binding proteins interact with genomic regulatory regions as a central mechanism to direct cell-specific transcriptional programs. Though initially considered a benign scaffold to package DNA, the nucleoprotein structures of chromatin are now recognized as integral to the accessibility of transcription factors to gene regulatory elements [1]. A relaxed euchromatin structure where the DNA template is loosely associated with histone proteins allows the transcriptional machinery of the cell to access and read the genetic code. This contrasts with transcriptionally repressive conformations where DNA is tightly associated with histones and buried within the chromatin. By influencing the chromatin-penetrating potential of transcription factors, the structural re-organization of chromatin underpins the selective activation and silencing of gene programs that give rise to an astounding array of cell types from a single source of genetic information. Just as importantly, chromatin dynamics underlie a cell's ability to manage environmental variations with rapid changes in gene activity [2].

Active and silent regions of the genome are distinguished by small chemical modifications to histones and the DNA itself that markedly shape the chromatin architectures by recruiting or precluding other factors that remodel the histone-DNA complex. Because the chemical tags can regulate changes in gene expression independently of the underlying DNA sequence, they are called epigenetic (literally in addition to genetic). These molecular signatures have an enormous capacity to control the functional state of chromatin and transcriptional activity of genes encoded therein for both dividing and terminally differentiated cells. Chromatin modifications can be influenced by various environmental factors, hence epigenetic grammar contextualizes the language of the DNA code [3].

The importance of this environment-epigenome axis is emerging

for many disease states [4-8]. This is exemplified by recent large clinical studies of diabetes. Indeed the pathogenesis of type 2 diabetes has a particularly strong association to environmental factors that are proposed to alter the chromatin landscape. Likewise, diabetes-associated perturbations in metabolism and hemodynamics are known to influence the development of vascular complications by epigenetic modifications. Moreover, some of these chromatinized changes are implicated in the phenomenon of metabolic memory in type 1 and type 2 diabetes, where antecedent periods of hyperglycemia drive persistent vascular complications many years after blood glucose control is achieved [9]. To this end, epigenetic profiling of circulating blood monocytes has revealed a persistent histone acetylation signature at genes implicated in diabetes complications that was closely associated with glycemic history in patients with type 1 diabetes [10].

While numerous enzymes and specific modifications have been described, defining the cell's ability to sense the variety of diabetic signaling cues at the chromatin level remains an important challenge. Precisely how is information communicated to the orchestra of factors controlling the chromatin landscape? How does the epigenetic machinery interact with transcription factors to control gene expression? Who regulates the genome regulators?

Oxidative stress alters the chromatin landscape of vascular cells

The functional relationship between chromatin architecture and changes in gene expression conferred by chronic and prior hyperglycemia has proven to be an important avenue of investigation for explaining persistent vascular complications of diabetes. We previously described the critical role of H3 histones lysine 4 mono-methylation (H3K4m1) in the high glucose-mediated transcriptional activation of human endothelial NFκB-p65 (encoded by the RELA gene), a key pro-inflammatory transcription factor that regulates the expression of genes implicated in inflammation associated with vascular complications of diabetes [11,12]. Moreover this specific chromatin signature, written by the Set7 lysine methyltransferase, persisted for up to 6 days in normal glucose conditions, suggesting it could confer future cell memories. The clinical relevance of these seminal *in vitro* findings was recently validated in the peripheral blood mononuclear cells of a cohort of patients with type 2 diabetes [13].

Identification of the methyl writer in the chromatinization of glucose signaling cues raised a new question. How are changes in ambient glucose transmitted to Set7? Indeed Set7 is mobilized to the nucleus with increasing glucose concentration [14]. Mitochondrial overproduction of superoxide has long been known to initiate many hyperglycemia-induced mechanisms related to the pathogenesis of diabetic complications [15]. Accordingly, the up-regulation of RELA induced by transient hyperglycemia was abolished by overexpression of either uncoupling protein-1 (UCP-1) or manganese superoxide dismutase (MnSOD), both of which prevent hyperglycemia-induced superoxide production [11]. Further, Paneni and colleagues identified epigenetic changes driving up-regulation of the mitochondrial adapter protein and critical mediator of oxidative stress $p66^{Shc}$ in vascular endothelial cells cultured in high glucose conditions [16]. The resulting superoxide production activates PKCβII, which in turn maintains elevated $p66^{Shc}$ levels, ultimately stimulating and sustaining epigenetic changes by enzymes such as Set7 [17]. While this mechanism has the capacity to explain the sustained legacy of hyperglycemia in diabetes, it raises further challenges in identifying how high glucose signals to the epigenetic machinery regulating $p66^{Shc}$ expression.

Chromatin modifiers interact with transcription factors

Large datasets reveal striking overlaps between transcription factor binding sites and chromatin modifications [18]. Co-regulatory interactions between transcription factors and chromatin-modifying enzymes may at least partly account for this co-localization [19]. Set7 was shown to be co-recruited with TAF10 to activating gene promoters [20]. In fact, along with its role in methylating histones, Set7 interacts with numerous transcription factors across various cell types, often promoting methylation reactions on regulatory lysine residues at the surface of the transcription factor [21].

Our recent characterization of human vascular endothelial cells depleted of Set7 revealed widespread changes in gene expression across numerous pathways associated with vascular function that were only partly explained by changes in H3K4m1 at promoters and distal enhancer regions [22]. By intersecting the transcriptome profile with publicly available datasets, we identified strong associations between deregulated genes and six transcription factors previously described as Set7 methylation substrates: NFκB, STAT3, IRF1, p53, ERα, and TAF7 [21]. In addition, many deregulated genes were associated with numerous transcription factors not previously connected with Set7 function. By applying a consensus formula derived from Set7 substrates previously used to accurately predict several biochemically validated *in vivo* non-histone substrates [23], we predicted that Set7 post-translationally regulates transcription factors associated with vascular endothelial expression through the presence of Set7 amino acid methylation motifs. Amino hydrophobicity analysis indicated most predicted sites to be accessible to post-translational modification. Further, *in vitro* peptide methylation assays suggest that Set7 can indeed modify a predicted site on the STAT1 transcription factor, demonstrating the predictive value of our method to identify novel candidate substrates to analyze *in vivo*. Further characterization of putative substrates identified in these studies has the capacity to identify not only functional modulation of transcription factors, but also substrate-driven co-recruitment of the enzyme to specific promoters to potentiate H3K4m1 enrichment.

Like the regulatory function of acetylation, lysine methylation has emerged as an important post-translational modification for modulation of transcription factors [24]. Our novel method of mapping transcriptional changes to transcription factors for the identification of putative substrates with strong associations

to functional changes is applicable to substrate prediction for other broad-substrate histone modifiers [22]. Both the histone- and non-histone-modifying activities of epigenetic enzymes are important considerations for future strategies of pharmacological targeting in the clinic.

Chromatin modifiers regulate each other

A growing body of evidence points to post-translational modifications as regulators of chromatin modifying enzymes themselves [2]. The prevalence of these modifications suggests a highly ordered and dynamic network of components capable of writing, reading, and erasing modifications at both the chromatin template as well as each other.

Counted among the expanding catalogue of experimentally validated methylation substrates of Set7 are SUV39h1 and DNMT1 - enzymes that methylate histones (at a distinct site to Set7) and DNA respectively. Methylation at lysines 105 and 123 by Set7 impairs the repressive histone modifying capacity of SUV39h1 [25], whereas the stability of DNMT1 is regulated by Set7-mediated lysine methylation [26]. Similarly Set7 also methylates multiple lysines on the p300/CBP-Associated Factor (PCAF) histone acetyltransferase [27]. A single epigenetic enzyme therefore has the ability to control many chromatin modifications. Mapping the inter-enzyme modification network of epigenetic regulators has an enormous capacity to increase our understanding of gene regulation and further raises important considerations for therapeutic strategies aimed at editing the epigenome.

Conclusion

Immense interest surrounds efforts to modulate gene expression, to restore the activity of silenced genes or attenuate unscheduled gene expression. However, the complexity of gene regulation is vast and researchers are only starting to gain an appreciation for the biochemical determinants and genome-wide inter-connectivity of epigenetic enzymes and transcription factors. Using the example of Set7, we have described recent observations that expand the understanding of chromatin biology beyond the immediate histone methyl-writing event. Indeed this is just a scratch on the surface and further studies are required to completely understand this important enzyme. Similar questions of biochemistry and interactivity remain for many other classes of chromatin modifiers considered useful in the clinic, including methylases, demethylases, acetylases and deacetylases, as well as protein components responsible for reading the chromatin mark such as bromodomains. While chromatin modifications can be informative of gene regulation at specific loci, the challenge of understanding their cell-specific function remains unmet. Myeloid-specific genetic deletion of histone deacetylase 3 is associated with stable atherosclerotic plaques [28], whereas deletion of the same enzyme in endothelial cells enhances atherosclerosis in mice [29].

More recently emerged concepts could offer further insight into epigenomic regulation. For example several intermediates of cellular metabolism are critical substrates for chromatin modifying enzymes. Fluctuating levels of these metabolites could therefore signal for continual adjustment and contextualization of gene expression (recently reviewed [2]). In addition long non-coding RNA molecules could play a role in the localization of chromatin signatures. By simultaneously recruiting two different histone modifiers to the chromatin – one a writer and the other an eraser of histone methylation – the HOTAIR long non-coding RNA facilitates the coordinated addition of a repressive modification and removal of an activating one to silence specific genes [30].

Technological and scientific advances have rapidly expanded the field of epigenetics to the point where chromatin modifiers are seriously considered as therapeutic targets for numerous diseases. The challenge for the next decade is to develop a comprehensive understanding of the biochemical and molecular events controlling the genome's regulators.

Conflicts of Interest

The authors declare no conflicts of interest.

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