

Epigenetics and Hyperglycemic Memory

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Epigenetics relates to stable and heritable patterns of gene expression that do not involve changes in DNA sequence. Cell identity is exquisitely controlled by specific gene expression patterns. These persisting regulatory events associated with gene expression changes can be attributed to antecedent information, suggesting that previous or transient life experiences influence successive epigenetic information. Analogous to Hamlet's soliloquy the "thousand natural shocks that flesh is heir to," so too exists the instinctive continuity of epigenetic information transmitted from generation to generation. Indeed, each cell type is the beneficiary of thousands of committed modifications on the epigenome, which instruct critical nuclear functions in processes involving development, cell proliferation and cellular responses to the environment. Although we are beginning to understand the flow of epigenetic information that governs gene activity patterns or the formation of instructive chromatin polymers, we still do not know the regulatory machineries that segregate permissive from suppressive chromatin landscapes. For some time now, dogma decreed that inactive regions of the genome were deficient for binding of transcriptional co-activators and were generally thought to be unimportant, while at the other end of the spectrum, active gene sequences were generally assumed to be deficient for co-repressor binding. In the laboratory, not only are we specifically interested in studying these extremes, but also interested in defining the series of determinants required for diverse transcriptional regulatory activities in order to understand human health and disease.

Histone Tail Modification and Gene Expression

A central mechanism in gene regulation resides in the histone-protein packing of genomic DNA into chromatin. Histones are the most abundant proteins associated with eukaryotic DNA. The basic unit of the nucleosome is 146 base pairs of DNA wrapped around a histone octamer (1). Histone N-terminal tails protrude from the nucleosome and are rich in positively charged basic amino acids (such as lysine), which interact with the negatively charged phosphate groups in DNA to establish intra- and inter-nucleosome interactions. Nucleosomes interact to create a highly compact structure (heterochromatin) that limits access of genomic DNA to transcription factors, thereby repressing gene expression. N-terminal tails of histones are targets for enzymes that mediate specific post-transcriptional modifications, particularly on lysine and arginine residues, which together form a 'histone code' that governs chromatin structure and function (1,2). Histone acetyltransferases (HATs) acetylate specific lysine residues to stimulate gene expression by neutralising the positively charged lysine residue, resulting in destabilisation of the histone-histone and histone-DNA interactions that limit access of transcription factors to DNA. The effect of HATs

is counteracted by histone deacetylases (HDACs), which function as transcriptional co-repressors. There are at least 17 HDACs and two predominant classes: class I HDACs that are ubiquitously expressed and class II HDACs that are present in the heart, skeletal muscle and the brain. In addition to histone tail acetylation and deacetylation, methylation of histone lysine has also emerged as a significant epigenetic mark that can format chromatin structure and function. The diverse post-translational modifications to histone termini reveal an extraordinary level of regulation that further emphasises the importance of epigenetic modifications influencing chromatin organisation and transcriptional control.

Histone lysines can be methylated by a group of enzymes called histone methyltransferases (HMTases) (3) (Fig. 1). Members of this enzyme family contain a conserved SET (Su(var)3-9, Enhancer of Zeste, Trithorax) domain, which is flanked by cysteine-rich regions. The conserved chromodomain modules found in chromatin-associated proteins related to heterochromatin proteins recognise methylated lysines. Although methylation of histones H3K9, H3K27 and H4K20 function as a repressive mark, not all lysine methylation appears to be a signal for the suppression of transcription (4). It has been only recently demonstrated that methylation of histones H3K4, H3K36 and H3K79 are associated with active genes (3).

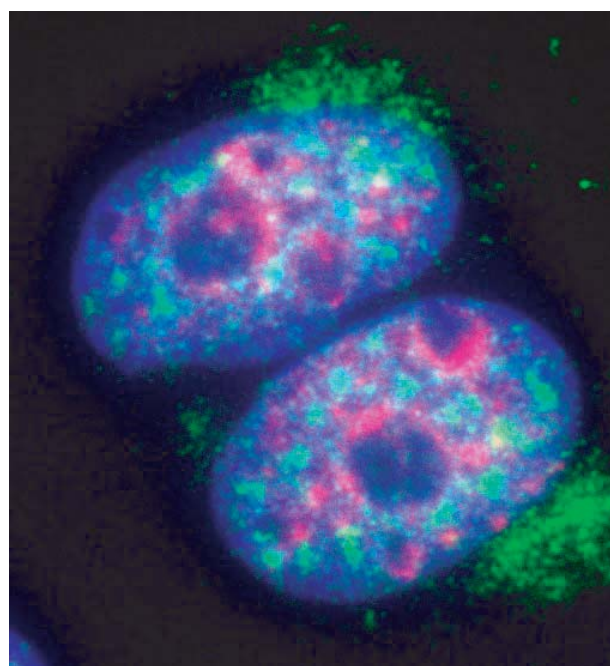


Fig. 1. Subnuclear distribution of the putative histone methyltransferase (green) and HP1 (heterochromatin-associated protein; red) proteins in human microvascular endothelial cells. DNA was stained with DAPI (blue).

DNA Methylation

Another key epigenetic regulator is DNA methylation (2,5,6). This mechanism plays a key role in the regulation of cell division and differentiation, DNA replication, DNA repair and gene transcription. DNA hypermethylation is also regarded as a critical mechanism in tumorigenesis through silencing of tumour suppressor genes whilst hypomethylation is associated with certain types of autoimmune diseases. DNA hypermethylation dominantly silences gene transcription by either blocking transcription factors to bind with DNA or by facilitating binding of methylated CpG-binding proteins (MBDs), which are biochemically linked with HDAC repressor activity (7). DNA methyltransferases (DNMTs) can also function as transcriptional suppressors in collaboration with HDACs (5). Both DNMT1 and DNMT3a can form complexes with HDACs and have the capacity to repress transcription independently of their methylation capabilities. Synergistic interactions of histone deacetylation and DNA methylation have also been demonstrated using specific inhibitors *in vitro* and *in vivo*. There is strong genetic evidence that DNMT cooperativity and CpG methylation are critical in gene silencing and suggests co-regulatory molecules such as MeCP2/MBD proteins do not act alone in repression. What were once considered separate pathways can now be viewed as complementary by their very association with histone deacetylase complexes.

Chromatin Disruption and Methylation

One of the challenges in the field is to understand how DNMTs target regions of the genome for methylation. Recent studies attempt to address this question by examining the mutant oncogenic transcription factor PML-RAR and its role in methylation dependent gene silencing (8). PML-RAR functions as a transcriptional repressor by its association with HDAC. PML-RAR causes hypermethylation of the RAR β 2 gene, which can be alleviated by the demethylating agent, 5-aza-deoxycytidine (5adC). DNMT1 and DNMT3a interact with PML-RAR protein and are enriched on the RAR β 2 promoter. Inhibitors such as 5adC or the HDAC inhibitor, trichostatin A, can partially relieve silencing whereas the combination results in robust RAR β 2 expression, typical of methylation-dependent transcription systems (9-11). These intriguing findings indicate that the targeting of DNMT enzymes to gene sequences can be regulated by chromatin determinants that include the methylation machinery. Colleagues in the field recently described that chromatin disruption might account for DNA methylation changes (12). Lymphoid-specific helicase is a member of the SNF2 subfamily that has conserved ATPase/helicase motifs (13) and shows strong sequence homology with human SNF2h and Brg1. Interestingly, DNA hypomethylation mutants isolated in a genetic screen of the flowering plant *Arabidopsis* showed the *ddm1* locus is responsible for a decrease in DNA methylation (14). The amount of DNA methylation was reduced by as much as 70% in these mutants and the *de novo* methylation rate was severely compromised even though *ddm1* is not a methylase, but rather, a member of the SWI2/SNF2 family (15). This raises important questions about chromatin accessibility.

ATP-Dependent Chromatin Remodelling by SWI/SNF

The functional state of chromatin can be regulated by at least three different strategies of which we have already discussed two: differential association of non-histone proteins such as the methyl-CpG binding protein family and covalent modification of the histones themselves. The third category involves ATP-dependent movement of chromatin. The prototype of this remodelling machine is the conserved human SWI/SNF, a multi-subunit complex composed of Brm and Brg1. It is established that yeast SWI/SNF, Brahma and human SWI/SNF activity can lead to the relief of repression (see reviews 16,17). It is now becoming clearer that transcriptional repression involves the complex interaction of several different components. The inability of DNA demethylation inhibitors to relieve transcriptional repression on hypermethylated sequences regardless of hyperacetylation of histones led us to directly investigate the involvement of remodelling and suggests the co-repressor complex is not alone in repression (9).

Cardiovascular Disease, Myogenesis and Hypertrophic Signalling

Recent evidence reveals the importance of the HAT/HDAC mechanism in myogenesis and hypertrophic signalling (18-20). HDAC functions as a repressor for myogenesis by its binding to some transcription factors, including MyoD, MEF2 and serum response factor, all known to play pivotal roles in myogenesis, and such inhibition can be cancelled by HDAC phosphorylation (18). Recent studies have shown that histone deacetylation inhibits hypertrophic signalling mediated by MEF2 (18,19). Class I and class II HDACs contain a unique 18 amino acid motif through which they bind with all MEF2 family members. Such association prevents MEF2 acetylation by HATs and represses gene transcription. Phosphorylation of HDACs by Ca²⁺/calmodulin-dependent kinase recruits 14-3-3 to dissociate the HDAC-MEF2 complex. Hence, MEF2 is free to associate with and acetylates the nucleosome, activating expression of hypertrophic genes (19). Indeed, HDAC9-deficient mice experience severe hypertrophy after banding of the transverse aorta (19). Because histone acetylation is required for gene activation, it is thus expected that HDAC inhibitors would promote hypertrophic growth. In cultured cardiac myocytes, however, class II HDAC inhibitors unexpectedly suppress hypertrophy growth and stimulate expression of α -myosin heavy chain (20).

These findings strongly indicate a role for chromatin remodelling via various modifications in the modulation of gene transcription and hypertrophy signalling. Almost all efforts to understand myocardial gene control have focused on the role of transcription factors that control human transcriptional regulatory networks. Our understanding of transcriptional control in recent years has come from scientific advances that represent many disparate disciplines such as cancer genetics and epigenetics, remodelling of chromatin during human development and the prominence of epigenetic modifications in disease. Multidisciplinary approaches have emerged from different fields of research and reveal a complex model of gene control. More importantly, they forge together a foundation of cell

signalling and transcriptional regulatory circuit that serves as a model for understanding the epigenetic basis of human disease more generally. We now realise that the transcriptional response is part of an epigenetic program. However, it remains unknown whether an epigenetic program is involved in myocardial gene regulation during heart failure or the underlying molecular specificity that mediates a transcriptional response in heart disease. This has raised many important questions related to the epigenetic regulation in the heart. For example, how do the histone and genomic methyltransferase enzymes operate to achieve rapid and precise control of gene expression in response to physiological and pathological signals? What is the role of epigenetic factors in the up- or down-regulation of gene expression seen in hypertrophied and failing hearts? More importantly, what is the cause-effect relation of altered epigenetic mechanism in the failing heart?

Hyperglycemic Memory Despite Intensive Therapy

Hyperglycemia, associated with endothelial cell dysfunction and alterations in blood vessel growth, is the primary cause of vascular complications in diabetes (21). Vascular complications are the major source of morbidity and mortality in diabetes, affecting multiple organs. Furthermore, these complications often persist and may progress despite improved glucose control, possibly as a result of prior episodes of hyperglycemia. Results from the Diabetes Control Complications Trial (DCCT) and the subsequent Epidemiology of Diabetes Interventions and Complications (EDIC) study have revealed that the deleterious end-organ effects that occurred in both conventional and intensified glycemic control groups continued to operate more than five years after the patients had returned to usual glycemic control (22,23). These studies suggest that the injurious effects of exposure to high glucose levels persist for years after treatment, a phenomenon typically referred to as 'hyperglycemic memory'. In April 2003, the National Institutes of Health convened a special symposium 'Metabolic Imprinting and the Long-Term Complications of Diabetes Mellitus' on the 20th anniversary of the DCCT/EDIC studies to discuss potential explanations for this phenomenon. Although no data were specifically provided, a number of hypotheses were raised, including the significance of epigenetics per se as a potential mechanism linking the progression of diabetes with its complications. However, the precise mode of action whereby transient hyperglycemia induces persisting epigenetic changes that are specifically associated with gene activity still remains poorly understood.

Evidence from the landmark DCCT and UK Prospective Diabetes Study (UKPDS) shows that improved glucose control reduces the complications of diabetes (24-26). The current goal of diabetes therapy is to reduce time-averaged mean levels of glycemia, measured as hemoglobin A1c (HbA1c), in order to prevent diabetic complications (25). Indeed, results of these studies suggest that it is not solely the overall level of glycemic control, as reflected by HbA1c, that

determines the susceptibility and progression of diabetic complications. Recent work indicates that the amplitude of glycemic excursion may be an HbA1c-independent risk factor for diabetic complications, and that this may be due to the ability of acute hyperglycemia to increase the production of reactive oxygen species. It is increasingly appreciated that glucose is the major factor leading to these complications, based on studies performed in type 1 (DCCT) and in type 2 diabetes individuals where intensified glycemic control significantly reduced the development and progression of diabetic micro- and macrovascular complications.

It is now clearly evident that hyperglycemic memory, also known as metabolic memory, could have long-lasting effects. Indeed, the DCCT/EDIC outcome data demonstrate that despite similar levels of glycemic control, the original intensive therapy group continued to have reduced rates of complication development despite having similar levels of glycemic control to the conventional group over the five-year study. Hyperglycemic memory is a clinically important and unresolved problem in diabetes and its complications have raised the pertinent question: how could a finite period of good or poor glycemic control have such long-lasting effects? Collectively, the observations of these large studies support the concept that an early period of hyperglycemia can lead to sustained and long-lasting effects within the vasculature, ultimately resulting in organ injury and dysfunction (27). Indeed, the re-institution of good metabolic control for many months in diabetic animals does not easily reverse key pro-inflammatory markers such as NF κ B activity, further highlighting the deleterious and long-lived effects of transient and prior hyperglycemia (28).

DNA and histone methylation were previously considered to be fixed, resistant and not subject to dynamic change. Recent studies have, however, brought to light key experimental information that is changing the view of genetic rationalism. Data are now emerging that indicate that changes in methylation and other epigenetic processes are central to the processes of developmental plasticity and underpin the relationship between early life effects and nutritional status (29-32). These findings point towards the concept that previous and transient environmental stimuli can significantly influence successive generations (33) and as such, these epigenetic marks are reversible (34). Although these studies do not specifically explain the molecular machineries that regulate epigenetic modifications, we are beginning to understand and appreciate the increased significance of prior nutritional and metabolic status on genes and their modifications. These effects have been attributed to differences in the epigenetic information accompanied by chromatin structure. Primary observations from our laboratory indicate that transient hyperglycemia induces long-lasting activating epigenetic changes in pro-inflammatory markers. These results highlight the dramatic and long-lasting effects that short-term hyperglycemic excursions can have on vascular cells. We postulate that persisting activating events could be attributed to changes in epigenetic information accompanied by changes in chromatin structure and its

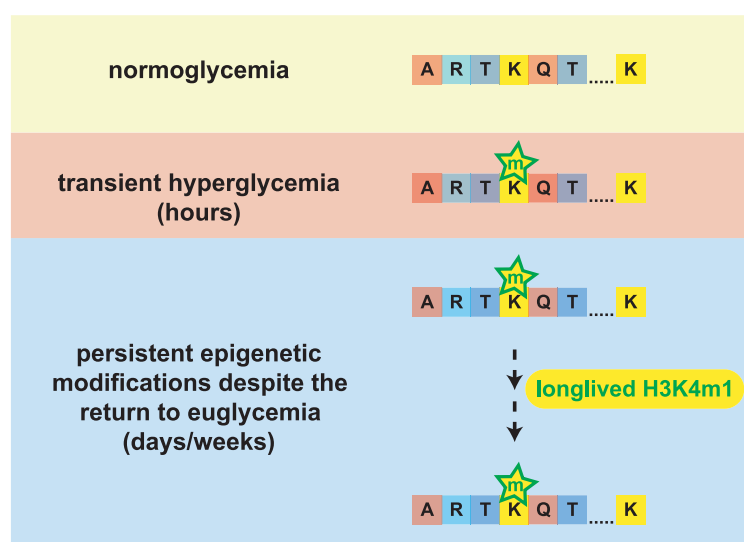


Fig. 2. Hyperglycemic memory and gene-activating epigenetic change.

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modification. Seeking the basis of this epigenetic persistence, we have screened for histone activation marks to reveal startling changes to H3 methylation that were maintained for at least six days when endothelial cells were returned to normoglycemic conditions, suggestive of epigenetic activation events that are maintained in subsequent cell generations (Fig. 2). Chromatin immunopurification analyses performed in our model of hyperglycemic variability indicate that epigenetic persistence of gene expression is maintained with the recruitment of histone methyltransferase enzymes to promoters. These novel findings indicate that transcriptional competence is linked with persisting epigenetic marks that are maintained when the endothelial cell is out of its acute hyperglycemic context.

Conclusion

The typical diploid human cell contains two sets of 23 chromosomes (22 pairs of autosomes and one pair of sex chromosomes), which equates to approximately six billion base pairs of genetic material. In order to functionally process the entire length of sequence, the genome is compacted by histones that form the chromatin fibre, which significantly reduces this length to almost 400 nm. Histone tails are subject to a variety of post-translational modifications that regulate chromatin structure and function. Epigenetics can be considered as genomic origami ('ori' meaning 'fold' and 'gami' meaning 'paper'). Epigenetics literally means 'above the genome', so if we consider origami paper to represent genomic material, then each fold or crease would represent a specific epigenetic mark on the genome. Critical to the faithful transmission of epigenetic information and gene expression is the temporal development of each fold or mark. The array and type of each epigenetic mark, akin to the number and combination of different folds, ultimately dictate the intricate origami designs. In a similar fashion, it is critical that we understand how the epigenome is carefully regulated and maintained not only for human development, but also increased understanding of epigenetic disease mechanisms.

