Redox Mediating Epigenetic Changes Confer Metabolic Memories

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It’s not always the case that it’s easy to forgive and forget, particularly when it comes to past memories. The concept of the legacy effect or hyperglycemic memory describes the deferred consequence of antecedent glycemic status on the development of diabetic complications. Anyone researching chronic hyperglycemia appreciates that glucose is still considered the major risk factor implicated in the development and progression of diabetic vascular complications. Now, the same can be concluded for transient hyperglycemia. Large clinical studies have demonstrated that prior glycemic control has a sustained benefit in reducing subsequent diabetic complications.1–4 The Diabetes Control and Complications Trial (DCCT) and the follow-up study, Epidemiology of Diabetes Interventions and Complications (EDIC) have determined that episodes of poor glycemic control can lead many years later to the long-term complications of diabetes.5,6 The DCCT study was designed to compare intensive versus conventional approaches to improve glycemic control and determine the effects of these regimens on the development and progression of vascular complications in patients with type-1 diabetes. Because the development of diabetic microvascular complications and cardiovascular disease takes time, the follow-up EDIC study was designed to investigate the long-lasting effects of intensive and conventional therapies. The findings of these extended studies show that early intensive intervention was more effective in slowing the development of diabetic complications and this was clearly evidenced with the benefit of 6.5 years of intensive therapy during the DCCT phase being sustained, the benefits continuing for at least 10 years after return to usual glycemic control. When taken together, these studies in type 1 diabetes as well as the more recent findings from the follow up of the United Kingdom Prospective Diabetes Study emphasize that prior periods of intensive glycemic control significantly reduces the major burden of diabetes, its vascular complications.7

Although the link between hyperglycemia and diabetic complications has been intensively studied, the interaction between gene and environment as a mechanism in the etiologic pathway of complex vascular disease remains poorly understood. Emerging evidence has identified a new research direction, specifically the importance of epigenetic modifications in conferring future metabolic memories. Mapping this new route of glycemic memory actually has an underappreciated history.8 The first glimpse into this phenomenon came from the work by Roy and colleagues.9 The authors reported that elevated gene expression, specifically fibronectin, could be induced by transient hyperglycemia in human endothelial cells and streptozotocin-induced diabetic rats which persisted even after the restoration to normoglycemia. At the time of that study, the underlying mechanisms regulating gene expression were not examined and for more than 2 decades remained poorly characterized.

Many years later histone modifying enzymes were identified and were shown to be associated with persistent gene expression, at least in part mediated by increased mitochondrial ROS (reactive oxygen species) generation.10 As well as being recognized as a histone methyl-writing enzyme11 distinct from non-histone substrates in models of hyperglycemia,12 knockout of the Set7 methyltransferase prevented glucose-induced expression of proinflammatory molecules, a prominent feature of vascular disease, particularly in the setting of diabetes. Perhaps the most obvious way in which cells of the vasculature react to hyperglycemic conditions is their redox-controlled response driving gene expression changes. Indeed, much subtler effects exist and one of these is the participation of chromatin modifying enzymes regulating the expression of nuclear transcription factor-κB, which is central to the upregulation of adhesion molecules and chemokines, which is associated with endothelial cell dysfunction. Despite the intense interest of oxidative stress as a regulatory mechanism linking memory with hyperglycemia,13 understanding the downstream targets that mediate diabetes associated epigenetic modifications will be critical to preventing or treating diabetic complications.

The work of Brownlee has shown that intracellular hyperglycemia induces mitochondrial superoxide production and is considered to play a critical role in the pathogenesis of diabetic complications.14,15 The overproduction of ROS is considered to be one of the earliest molecular events in the activation of mechanisms relevant to glucotoxic end organ damage, which include activation of the polyol pathway and increased hexosamine flux. Other key pathways include the activation of the intracellular signaling molecule protein kinase C-β (PKC-β) and nonenzymatic glycation of intracellular and extracellular proteins causing the increased formation of advanced glycation end products as well as the increased flux of glucose metabolism causing oxidative damage due to ROS overproduction. Indeed, vascular endothelial cells are vulnerable to damage because glucose metabolism in these cells is tightly associated with...
circulating glucose levels. The signaling cues that activate these pathways are self-maintaining and cause persistent ROS generation, independent of circulating glucose levels and thus are ideal candidates for the vicious cycle of metabolic memory. A key challenge to understanding persistent gene activation events is explaining how gene expression in vascular cells is altered in response to transient exposure of extracellular stimuli such as glucose. Strikingly, as characterized in both human and mouse aortic endothelial cells, the Set7 methyltransferase enzyme acts as a hyperglycemic sensor and is associated with distinguishable histone and nonhistone gene-regulatory events.

Once again, the results of these experiments emphasize the importance of intracellular glucose metabolism conferring distinct chromatin modifications. Not only is gene expression associated with the legacy of hyperglycemia, but reducing mitochondrial superoxide production or superoxide-induced α-oxoaldehydes such as methylglyoxal also prevents gene-activating chromatin modifications. However, this explains only part of the complex gene regulatory events that are associated with oxidative stress in mediating diabetes-associated epigenetic changes.

In this issue of Circulation Research, Paneni and colleagues further expand on the role of oxidative stress and specifically the mitochondrial adapter p66shc which is a downstream target of PKC-βII, a known mediator of diabetic complications. Mitochondrial p66shc is a lifespan adapter protein in mammals, which regulates ROS metabolism and apoptosis. The endothelial cell is considered a key vascular cell capable of sensing changes in glucose concentrations because of its location in the blood vessel wall. These endothelial cells are viewed as critical in transmitting signaling cues to underlying layers of the vessel wall by altering gene expression patterns. The authors demonstrate a role for the cytoplasmic signal p66shc protein in hyperglycemic memory. Following transient exposure to high glucose persistent p66shc upregulation is associated with continued ROS production in human vascular cells. PKC-βII belongs to a large family of PKC isoforms that is specifically activated and translocated by hyperglycemia. In contrast, transient hyperglycemia did not alter expression of other PKC isoforms such as PKCα, βI, γ, and δ. The importance of this PKC-βII isoform in diabetic complications has been previously investigated in experiments performed in PKC-βII isom knockout mice as well as in mice treated with the PKC-β inhibitor, ruboxistaurin. The identity of the molecule that mediates the interaction between PKC-βII upregulation and phosphorylation of eNOS has remained elusive. To investigate the role of PKC-βII, the selective inhibitor CGP 53353 was added at the time of glucose normalization. Phosphorylation of p66shc was abolished using the inhibitor suggesting that the activation of PKC-βII/p66shc persists even after restoration to normal glucose levels. Specifically, high-glucose led to the phosphorylation of eNOS at the Thr-495 residue, which leads to reduced eNOS enzymatic activity (see Figure). The restoration of normal glucose levels reversed these effects. But how might PKC-βII/p66shc activation persist and lead to endothelial dysfunction?

To test this, the authors assessed if gene-activating epigenetic changes were associated with glucose induced p66shc expression. Indeed, transient hyperglycemia promoted the persistent activation of p66shc gene expression via CpG demethylation of the promoter. Rather than use the more conventional approach of bisulfite sequencing, Paneni et al chose methylation sensitive restriction enzymes and qPCR to analyze DNA methylation changes at the p66shc promoter. They confirmed that DNA methylation of the p66shc promoter was inversely correlated with gene expression. To further define the methylation status of the p66shc promoter following normoglycemia, they showed that hypomethylation was maintained despite glucose normalization. To show that DNA methylation was inversely associated with the hyperacetylation of histones, the authors checked by protein blot that histone H3 was hyperacetylated and correlated with expression of the H3 acetyltransferase, GCN5. Transient hyperglycemia not only increased overall H3 acetylation but this particular histone modification also persisted during subsequent incubation at physiological glucose levels. These results are indeed intriguing and raise further questions as to whether hyperacetylation of the p66shc promoter is important for persistent endothelial dysfunction. Although this remains to be demonstrated and was not addressed specifically in the report, recent mapping of aortic endothelial cells for histone H3 acetylation suggests a fine balance exists between persistent gene expression patterns and genomic methylation. Moreover, restoration of normal glucose levels could indeed regulate long-lasting proinflammatory gene expression patterns that are characteristically observed in diabetic vasculature. Although once thought to be too subtle to affect CpG methylation or chromatin modification states, the immediate glucose-mediated transcriptional response could very well have long-term regulatory consequences. These epigenetic changes are now widely regarded to be dynamically regulated (Figure).

There are many ways to monitor the onset of glucose-mediated gene expression, with Paneni et al initially using in vitro models to define the regulatory events underlying hyperglycemic memory. To validate the in vivo relationship between p66shc and persistent endothelial dysfunction as a result of glycemia, the authors examined diabetic mice treated...
for 3 weeks with insulin to show that the restoration of normoglycemia did not revert diabetes associated p66shc activation. Because activating p66shc phosphorylation at Ser-36 was increased and consistent with translocation into the mitochondria, the authors hypothesized that this was linked to ROS-mediated effects and NO bioavailability. Indeed, when p66shc was silenced by RNA interference, oxidative stress was blunted as well as suppressing persistent endothelial dysfunction and vascular apoptosis.

The field of metabolic memory has advanced with the discovery that ROS mediated changes could confer a legacy effect. Oxidative stress is now thought to mediate diabetes-associated chromatin modifications with genomic methylation dynamically coordinated to regulate gene expression. Although these studies highlight the importance of antecedent glucose control and the persistent activation of the PKC-βII/p66shc signaling pathway, it remains to be determined as to the other physiological targets. Mapping the epigenome will provide clues to understanding the vast number of gene targets that are memorized in response to prior episodes of hyperglycemia despite restoration of normal glucose levels. This challenge is further complicated by the diversity of chemical variations to the histone tail as well as the potential effect on genomic methylation. Although an individual’s genetic sequence varies little from cell to cell, epigenomic diversity adds a whole new meaning to the long-standing concept that not all endothelial cells are alike. The development of diabetic complications takes years, but it is difficult to imagine how epigenetic pathways change with time. As ROS modulates signaling cascades that are important to hyperglycemic memory in the vasculature in diabetes, understanding the role of oxidative stress in gene regulation might also help us to identify therapeutically relevant enzymes implicated in chromatin structure and function. In our attempts to understand the consequence of glucose in terms of its effect on the complications of diabetes, we now appreciate that time changes metabolic memory and the persistence of epigenetic change steals time.

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