SIRT1, p66^{Shc}, and Set7/9 in Vascular Hyperglycemic Memory
Bringing All the Strands Together

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Diabetes represents a ramping public health issue in the Western world owing to overnutrition and reduced physical activity coupled with genetic susceptibility (1,2). Nowadays, obesity is a major determinant of insulin resistance, which results in compensatory hyperinsulinemia with subsequent impairment of insulin secretion and rise of blood glucose levels. Over the last 30 years, the detrimental effects of hyperglycemia on the vessel wall have promoted major research efforts leading to the understanding that high glucose decreases availability of endothelium-derived nitric oxide (NO) and triggers vascular inflammation via an array of mechanisms involving the overproduction of reactive oxygen species (ROS) (3,4). Although the identification of different signaling pathways has opened several windows of opportunity to prevent diabetic vascular disease, we are still far from having found a thorough and, most of all, effective approach against cardiovascular burden of diabetes. Indeed, recent clinical trials have shown that normalization of blood glucose failed to reduce cardiovascular outcomes in the diabetic population (5–7). It is noteworthy that intensive glucose-lowering therapy in these trials was started after a median duration of diabetes ranging from 8 to 11 years (8). By contrast, glucose-lowering treatment of patients with new-onset diabetes was shown to be beneficial (9–12). These findings hint that early preservation of physiological metabolic environment is crucial for interfering with the natural history of diabetic vasculopathy. In this Perspective, the landmark Diabetes Control and Complications Trial (DCCT) and the follow-up study, Epidemiology of Diabetes Interventions and Complications (EDIC), demonstrated not only that intensive glycemic control in subjects with type 1 diabetes reduced the risk of microvascular complications but also that episodes of poor glycemic control can lead many years later to the long-term complications of diabetes (9,12). More recently, the 10-year posttrial monitoring follow-up of the UK Prospective Diabetes Study (UKPDS) study showed that early treatment of hyperglycemia significantly reduced the risk of myocardial

infarction, diabetes-related deaths, and all-cause mortality (10). Collectively, these clinical observations imply that hyperglycemic environment may be remembered in vascular tissues.

HYPERGLYCEMIC MEMORY

The persistence of hyperglycemic stress despite glucose normalization has been defined as "hyperglycemic memory" (13,14). This emerging concept strengthens the importance of early glycemic control and may explain why diabetic cardiovascular complications progress even in the presence of optimal glycemic control. The initial skepticism toward the concept of hyperglycemic memory, considered vague and not supported by solid evidence, has gradually given way to a growing interest in unmasking the underlying mechanisms. This phenomenon was initially described in mice with streptozotocin-induced diabetes, where normoglycemia restoration did not revert the expression of fibronectin mRNA in the kidney cortex, which was elevated for several weeks even after the maintenance of near-normal glucose levels by exogenous insulin administration (15). The putative mechanisms were investigated in human endothelial cells exposed to hyperglycemia followed by normal glucose restoration. This experiment revealed that cells previously exposed to high glucose continued to display elevated expression of fibronectin and collagen IV despite subsequent normalization of glucose concentration in the media (15). Other investigations demonstrated the irreversibility of microvascular damage also in the diabetic retina (16). More recently, it was postulated that ROS may be critically involved in the persistence of hyperglycemic stress in endothelial cells and experimental diabetes (17–19). This concept is in line with the notion that ROS generation plays a leading role in the development of diabetes complications (4). Mitochondrial accumulation of ROS as a result of hyperglycemia activates major pathways involved in the pathogenesis of cardiovascular complications including polyol pathway flux, increased production of advanced glycation end products (AGEs), protein kinase C (PKC) activation, and the hexosamine pathway (4). Although ROS are upstream molecules regulating a number of detrimental pathways in the vessels, we are still far from understanding the mechanisms responsible for persistent changes of gene expression despite restoration of normoglycemia in the setting of diabetes. Ihnat et al. (18) found that oxidative stress markers and upregulation of pro-oxidant enzymes, namely PKCβ and NAD(P)H oxidase, persist after restoring normoglycemia in human endothelial cells previously exposed to high glucose concentrations. Accordingly, only diabetic patients treated with a combination of insulin and vitamin C at high doses showed an improvement of the

brachial artery flow-mediated vasodilation (20). Therefore, identification of ROS-generating machinery is the real challenge to reverse hyperglycemic memory and, eventually, prevent the development of cardiovascular complications.

COMPLEX SCENARIO INVOLVING EPIGENETIC CHANGES

A better understanding of the pathways leading to hyperglycemic memory may contribute to designing mechanism-driven strategies to blunt vascular damage and to improving cardiovascular outcome in subjects with diabetes. Although several studies have suggested that ROS plays a crucial role, only in most recent years have the molecular mechanisms of hyperglycemic memory been explored. These findings unravel a complex scenario of epigenetic changes that modulate transcription of ROS-generating and proinflammatory genes. Overproduction of ROS, in turn, maintains detrimental vicious cycles responsible for persistent vascular stress. Here, we provide a unifying context for the different pathways involved. While available knowledge supports single molecules as key mediators of this process, we proposed one intricate molecular pathway linking together the molecules implicated in chromatin remodeling, ROS generation, and vascular inflammation. Targeting individual components of this deleterious cascade may be the most promising option to dampen oxidative stress, reverse hyperglycemic memory, and, hence, prevent cardiovascular complications.

SET7/9 METHYLTRANSFERASE DRIVES PERSISTENT VASCULAR INFLAMMATION

In the last few years, several studies have identified critical molecular determinants of vascular hyperglycemic memory. Epigenetic mechanisms have been postulated and considered in metabolic memory only recently. Persistent epigenetic changes caused by hyperglycemia-induced mitochondrial ROS production have been advocated as the driving force underlying the progression of diabetic vasculopathy (21). Specifically, DNA methylation and histone modification represent a major breakthrough in understanding changes in gene expression occurring in disease states (21,22). Hypomethylation of CpG dinucleotides at the promoter region is generally related to enhanced gene activation. Promoter methylation interferes with transcription factors and facilitates the binding of methyl-CpG–binding domain proteins, which in turn recruit histone deacetylases, thereby repressing gene transcription (23). Methylation may also occur at lysine residue in the histone tail (24). This is a remarkably complex process, involving mono-, di-, or thrimethylation, which often clusters within specific regions resulting in the organization of chromosomes into distinct structural and functional domains (23). This process may lead to an open chromatin and gene activation rather than repression, as reported for CpG promoter methylation.

In 2008, El-Osta et al. (25) found that transient hyperglycemia in aortic endothelial cells was able to induce long-lasting activating epigenetic changes in the promoter of the nuclear factor κB (NF-κB) subunit p65. This epigenetic deregulation explains persistent *p65* gene transcription and subsequent overexpression of the inflammatory genes monocyte chemoattractant protein-1 (*MCP-1*) and vascular cell adhesion molecule-1 (*VCAM-1*) after 6 days of glucose normalization (25). Interestingly, normalization of

mitochondrial superoxide production dislodged the epigenetic markers at p65 promoter, clearly indicating that ROS still remain the upstream key molecules involved in the pathophysiology of diabetic vascular disease despite glucose control. Indeed, hyperglycemia-dependent ROS production was responsible for monomethylation of histone 3 at lysine 4 amino residue (H3K4 m) by the mammalian methyltransferase Set7/9. Methylation of H3K4 is a critical posttranslational modification favoring gene transcription in mammals (26) and is associated with persistent vascular inflammation when such methylation occurs on histone 3 binding proximal promoter region of NF-κB subunit p65 (25,27) (Fig. 1). Interestingly, knockdown of Set7/9 prevented H3K4 m and, hence, glucose-induced upregulation of p65 as well as of *MCP-1* and *VCAM-1* genes (25). For the first time, this study demonstrated that overproduction of ROS leads to activation of key enzymes involved in chromatin remodeling and persistent transcription of inflammatory genes.

MITOCHONDRIAL ADAPTOR p66[Shc] DRIVES ROS-INDUCED VASCULAR MEMORY

With such a background, we recently investigated whether mitochondrial adaptor p66[Shc], a critical modulator of intracellular redox state, is the source of ROS-inducing hyperglycemic memory. The p66[Shc] adaptor protein functions as a redox enzyme implicated in mitochondrial ROS generation and translation of oxidative signals into apoptosis. Mice lacking *p66[Shc]* gene (*p66[Shc−/−]*) display prolonged lifespan and increased resistance to oxidative stress and are protected against hyperglycemia-induced endothelial dysfunction (28). High glucose levels lead to increased *p66[Shc]* gene transcription and protein phosphorylation, favoring mitochondrial translocation, cytochrome c oxidation, and subsequent ROS generation (29). The clinical relevance of p66[Shc] in the setting of dysglycemia is supported by the notion that *p66[Shc]* gene expression is increased in mononuclear cells obtained from patients with type 2 diabetes and correlates with plasma 8-isoprostane levels, an in vivo marker of oxidative stress (30). In human endothelial cells exposed to high glucose and aortas of diabetic mice, we recently found that activation of p66[Shc] by PKCβII was sustained even after returning to normoglycemia (29). Persistent p66[Shc] upregulation and its mitochondrial translocation were associated with ROS production, reduced NO bioavailability, and apoptosis. Interestingly, *p66[Shc]* gene overexpression was epigenetically regulated by promoter CpG demethylation and acetylation of histone 3 operated by acetyl-transferase general control nonderepressible 5 (GCN5). These experiments also showed that p66[Shc]-derived ROS production maintained PKCβII upregulation and PKCβII-dependent inhibitory phosphorylation of endothelial NO synthase at Thr[495], contributing to a detrimental vicious cycle despite restoration of normoglycemia (Fig. 1). Importantly, in vivo gene silencing of p66[Shc], performed at the time of glucose normalization, blunted ROS production, restored endothelium-dependent vasorelaxation, and attenuated apoptosis by limiting cytochrome c release, caspase 3 activity, and cleavage of poly(ADP-ribose) polymerase (PARP). These findings strongly suggest that the adaptor p66[Shc] is a critical source of mitochondrial ROS, and its downregulation clearly reverses the pathological features of hyperglycemic memory in vascular tissues. Indeed, p66[Shc] knockdown after normoglycemia restoration was

associated with downregulation of PKCβII and eNOS dephosphorylation at Thr[495], as well as reduced synthesis of the AGEs precursor methylglyoxal (29) (Fig. 1). This latter finding suggests that long-lasting p66[Shc] activation may account for increased AGEs turnover, which has been considered a key factor underlying the glycemic memory (14). Methylglyoxal readily reacts with arginine, lysine, and sulfhydryl groups of proteins and nucleic acids, inducing the formation of a variety of structurally identified AGEs, which exert an inhibitory effect on mitochondrial respiration (31). AGEs represent an important pathway in the alteration of vascular structure and function. Specifically, the AGEs pentosidine and carboxymethyl-lysine strongly correlate with indices of arterial stiffness in patients with type 1 diabetes (32,33). The clinical relevance of AGEs in the context of hyperglycemic memory is supported by the notion that an early glycemic control significantly reduced AGE levels in the DCCT trial, and this finding was paralleled by a consistent reduction of cardiovascular events (34). In this Perspective, recent evidence suggests that soluble forms of AGE receptors (sRAGEs) bind ligands including AGEs and can antagonize RAGE signaling in vitro and in vivo, thus prospecting an attractive therapeutic option to reverse hyperglycemic memory (35).

Collectively, these results are in line with seminal studies supporting the pivotal role of ROS in the pathogenesis of diabetes complications and suggest the perspective that, even when glucose levels have been normalized, elevated concentrations of mitochondrial superoxide maintain the activation of intracellular pathways involved in endothelial dysfunction, vascular inflammation, and apoptosis. The role of epigenetic modifications in this setting is confirmed by our observation that glucose-induced p66[Shc] overexpression is driven by decreased promoter methylation and increased histone 3 acetylation (Fig. 1). Indeed, at the time of glucose normalization siRNA-induced downregulation or pharmacological inhibition of acetyltransferase GCN5 reported p66[Shc] expression to control levels (29). Our study identified a specific molecule responsible for ROS generation and may assist in defining novel therapeutic targets to reduce the long-lasting deleterious effects of hyperglycemia on the vasculature. This aspect deserves attention, since available antioxidants only partially scavenge cellular ROS but do not target intracellular redox signaling. Such an assumption is confirmed by the negative results of major trials with oral supplementation of high-dose vitamins (36).

SIRT1 REGULATES p66[Shc] TRANSCRIPTION

A recent study reported that vascular $p66^{Shc}$ gene transcription may be the result of decreased promoter deacetylation due to the downregulation of class III histone deacetylase SIRT1 (37). Expression of $p66^{Shc}$ gene transcript and protein was significantly increased by different kinds of class III histone deacetylase inhibitors in human endothelial cells. Consistently, SIRT1 overexpression inhibited high glucose–induced p66[Shc] upregulation, whereas SIRT1 knockdown exerted opposite effects. Moreover, endothelium-specific SIRT1 transgenic mice had

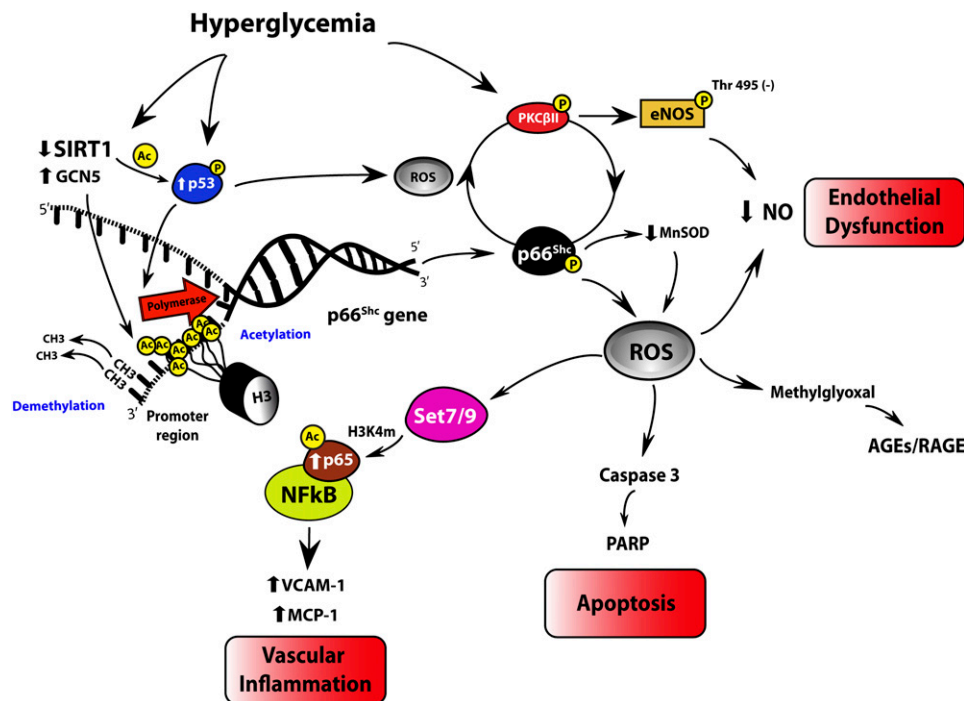FIG. 1. Signaling network of vascular hyperglycemic memory. Hyperglycemia via SIRT1 downregulation leads to acetylation of p53, NF-κB subunit p65, and histone 3 bound to p66[Shc] promoter. Activation of p53 leads to increased p66[Shc] transcription. In addition, glucose-induced GCN5 downregulation causes H3 acetylation and subsequent p66[Shc] transcription through chromatin remodeling. p53 protein as well as epigenetic-driven upregulation of p66[Shc] leads to persistent mitochondrial ROS production, which maintains hyperglycemia-induced PKCβII overexpression and PKCβII-dependent eNOS inhibitory phosphorylation at Thr[495] residue even after glucose normalization. p66[Shc] also downregulates MnSOD, further increasing ROS accumulation. These changes underlie endothelial dysfunction and apoptosis via reduced NO availability and activation of caspase 3 and PARP cleavage, respectively. SIRT1-p53-p66[Shc] networking via ROS production increases activity of the methyltransferase Set7/9, responsible for promoter monomethylation (H3K4 m) of NF-κB subunit p65 leading to its persistent transcription and subsequent upregulation of *MCP-1* and *VCAM-1* inflammatory genes. Ac, acetylation; p, phoshorylation; RAGE, receptor for AGEs.

blunted $p66^{Shc}$ gene and protein expression and improved endothelial function, as well as reduced accumulation of oxidative stress markers, compared with wild-type littermates (37). This study demonstrated that SIRT1 binds to the p66^{Shc} promoter (-508 to -250 bp) where it deacetylates histone 3, thus suppressing gene transcription of the mitochondrial adaptor (37). Hence, increased acetylation by GCN5 and decreased SIRT1-dependent deacetylation are the main epigenetic changes responsible for persistent p66^{Shc} overexpression in the vasculature despite glucose normalization (29,37) (Fig. 1). In addition, p66^{Shc} upregulation in human endothelial cells is associated with a significant reduction of the antioxidant enzyme manganese superoxide dismutase (MnSOD) (38,39). This latter finding suggests that p66^{Shc} is not only a critical source of mitochondrial ROS but is also involved in the downregulation of the scavenging enzyme, leading to unopposed ROS accumulation in the vascular endothelium (28) (Fig. 1).

In parallel with the notion that SIRT1 controls p66^{Shc} expression, a recent work reported that glucose-induced SIRT1 downregulation has a strong memory effect after restoration of normoglycemia in bovine endothelial cells (40). In this study, in vitro and in vivo SIRT1 overexpression associated with optimal glucose control was able to interrupt the memory of hyperglycemic stress via ROS normalization, thus suppressing NF-κB activation and cleavage of PARP (40). Overexpression of SIRT1 also restored hyperglycemia-induced dephosphorylation of LKB1 and AMP kinase (AMPK), two critical regulators of energy balance in mammalian cells (41). Interestingly, SIRT1 activation during subsequent normoglycemia restored normal expression levels of MnSOD (40).

These studies provide the molecular background to conclude that even after optimal glycemic control, persistent SIRT1 downregulation may account for p66^{Shc} overexpression leading to mitochondrial superoxide accumulation, reduced expression of scavenging enzymes, activation of NF-κB, and subsequent vascular dysfunction (Fig. 1 and Table 1). SIRT1 and GCN5 are the upstream regulators of $p66^{Shc}$ gene transcription by modulating the binding of histone 3 acetylation to p66^{Shc} promoter (Fig. 1). The pharmacological modulation of enzymes involved in the epigenetic regulation of p66^{Shc} is becoming an attractive therapeutic goal. In this regard, metformin through SIRT1 activation abolished oxidative stress and inflammation, reverting the pathological abnormalities in the retina of diabetic rats (40).

TUMOR SUPPRESSOR p53 IS A CRITICAL INTERMEDIATE BETWEEN SIRT1 AND p66^{Shc}

Another study found that glycemic control does not affect the upregulation of the tumor suppressor transcription factor p53 (42). Indeed, 7 days of glucose normalization after 14 days of prior hyperglycemia were not able to interrupt the activation of p53 as well as p53-induced proteins PTEN, p21, PUMA, and TIGAR. Persistent p53 overexpression was associated with ongoing ROS production and DNA damage, suggesting the important role of this transcription factor in the maintenance of oxidative stress (43,44). Molecular studies indicate that p53 might perform its senescence and proapoptotic functions by directly signaling the mitochondria and inducing cytochrome c release (44). Additional evidence supports the hypothesis that p53 may be part of a signaling pathway linking SIRT1 and p66^{Shc}. It was recently reported that

SIRT1 inhibition increases p53 acetylation and transcriptional activity (45,46). Moreover, p53 was found to transcriptionally regulate p66^{Shc} (47). Accordingly, downregulation of p66^{Shc} expression as well as inhibition of p53 function in mice restored impairment of acetylcholine-induced vascular relaxation and increased NO bioavailability (47). Taken together, these observations strongly suggest that p53 is a critical intermediate between the upstream regulator SIRT1 and its downstream target p66^{Shc} (Fig. 1 and Table 1).

In conclusion, SIRT1-p53- p66^{Shc} pathway might be responsible for self-maintenance of vascular damage after restoration of normal glucose levels. Indeed, recent evidence clearly demonstrated that these mediators remain upregulated despite glucose control (29,40,42). The activation of such a pathway, in turn, leads to increased mitochondrial superoxide production triggering Set7/9-related epigenetic changes on the promoter of NF-κB component p65 and subsequent vascular inflammation (Fig. 1 and Table 1). Furthermore, Set7/9 has been recently found to negatively regulate SIRT1 activity thus maintaining acetylation of p53 protein and activation of its downstream targets (48).

CLINICAL PERSPECTIVES

Since cardiovascular risk burden is not eradicated by intensive glycemic control, new mechanism-based therapeutic strategies are needed. The recent identification of epigenetically regulated genes in the setting of hyperglycemia may allow targeted approaches to reprogram these modifications (49). Plastic alterations of the chromatin are indeed responsible for the regulation of DNA-templated phenomena and may be amenable to pharmacological intervention. There are many examples suggesting the possibility of interference with gene expression by modulating acetylation and methylation of histone/DNA complexes (50,51). Folate treatment has shown to repress gene activation via increasing DNA methylation, and a correlation between homocysteine levels and such epigenetic modification was observed in healthy humans (51) (Fig. 2). Consistently, a recent work found that homocysteine stimulates p66^{Shc} transcription in human endothelial cells via specific CpG dinucleotides demethylation in the p66^{Shc} promoter (52). Interestingly, p66^{Shc} promoter CpG methylation was significantly reduced in peripheral blood leukocytes of patients with coronary artery disease and high plasma homocysteine levels, thus strengthening the relevance of p66^{Shc}-related epigenetic changes in the context of cardiovascular disease. On the other hand, SIRT1 activation by resveratrol improves vascular function via increasing NO availability and attenuates dyslipidemia and obesity-induced metabolic alterations in human subjects (53–55). SIRT1-dependent improvement of flow-mediated dilation can be partially explained by increased deacetylation of p66^{Shc} promoter as well as posttranslational and transcriptional regulation of endothelial NO synthase (eNOS) (37,56) (Fig. 2). By contrast, a recent study of 24 healthy obese men showed that resveratrol supplementation had no significant effects on insulin sensitivity, blood pressure, visceral fat content, systemic inflammation, or lipid oxidation (57). However, resveratrol has been tested only in a limited number of small human clinical trials of efficacy outcomes, and we are still far from understanding whether the persuasive results of experimental research may translate to the human setting.

TABLE 1
Experimental evidence linking detrimental pathways of vascular hyperglycemic memory

Publication	Year	Species	Main findings	Implications
Set7/9				
El-Osta et al. (25)	2008	Human/bovine	Hyperglycemia-induced ROS production causes upregulation of the mammalian methyltransferase Set7/9, responsible for lysine monomethylation of histone 3 bound to p65 promoter, leading to p65 upregulation and subsequent vascular inflammation. These epigenetic changes were not abolished by glucose normalization.	ROS are the upstream signaling molecules driving epigenetic modifications responsible for the persistence of hyperglycemic stress in endothelial cells.
Okabe et al. (27)	2012	Human/mouse	Response to hyperglycemia in vascular endothelial cells selectively involves the methyltransferase Set7/9, which regulates glucose-induced chromatin changes and gene expression via H3K4 m-dependent and -independent pathways.	Set7/9 is involved in persistent vascular gene expression despite glucose normalization and might represent a putative molecule contributing to hyperglycemic memory.
p53				
Kim et al. (47)	2008	Human/mouse	The tumor suppressor p53 is a critical regulator of p66^{Shc} transcription because of a p53 response element in the p66^{Shc} promoter. In mice, inhibition of p53 function strongly downregulates p66^{Shc} expression and improves angiotensin II–mediated endothelial dysfunction.	The tumor suppressor p53 is upstream of p66^{Shc} protein.
Orimo et al. (46)	2009	Human	In human endothelial cells, high glucose via decreased SIRT1 expression facilitates p53 acetylation contributing to vascular senescence and endothelial dysfunction.	SIRT1 modulates p53 at the posttranslational level.
Schisano et al. (42)	2011	Human	Transcriptional activity of p53 and p53-induced proteins PTEN, p21, PUMA, and TIGAR was not blunted by glucose normalization after prior hyperglycemia in human endothelial cells.	Persistent upregulation of p53 may contribute to ROS production, mitochondrial dysfunction, and apoptosis in this setting.
p66^{Shc}				
Paneni et al. (29)	2012	Human/mouse	In human endothelial cells and diabetic mice, glucose normalization does not revert hyperglycemia-induced p66^{Shc} upregulation. This phenomenon is regulated at the transcriptional level via decreased promoter methylation as well as increased histone 3 acetylation by GCN5. p66^{Shc}-derived ROS production, in turn, maintains a vicious cycle involving PKCβII.	The mitochondrial protein p66^{Shc} is a major driver of ROS-induced vascular hyperglycemic memory.
SIRT1				
Zheng et al. (40)	2012	Bovine/rat	Persistent SIRT1 downregulation accounts for sustained activation of LKB1/AMPK and NF-κB pathways even after normoglycemia restoration.	SIRT1 is a key determinant of vascular hyperglycemic memory.
Zhou et al. (37)	2011	Human/mouse	SIRT1 represses *p66^{Shc}* gene transcription via modulating the acetylation of histone 3 bound to its promoter. Endothelium-specific SIRT1 transgenic mice have blunted p66^{Shc} gene and protein expression, improved endothelial function, and reduced markers of oxidative stress.	SIRT1 regulates *p66^{Shc}* gene transcription in the vascular endothelium.
Liu et al. (48)	2011	Human	SIRT1 activity is negatively regulated by ROS-dependent methyltransferase Set7/9 thus leading to acetylation of p53 protein.	Set7/9 is a critical modulator of the SIRT1/p53 pathway.

Therefore, the activation of SIRT1 may be only a part of a much more complex scenario in which deacetylation of histones may play a role. In this Perspective, metformin, a widely used antidiabetes drug, restores SIRT1 expression/activity in the retina of diabetic rats (40). Indeed, it was demonstrated that 4-week metformin treatment, after 2 weeks of diabetes, significantly increased SIRT1 expression, preserved activation of its downstream targets LKB1 and AMPK, and blunted persistent ROS formation. Interestingly, metformin also suppressed NF-κB activation (40). Altogether, these findings are in agreement with the notion that SIRT1 is an upstream mediator regulating p66^{Shc} expression, ROS formation, and epigenetic-driven vascular inflammation (Fig. 1). Based on these experimental findings,
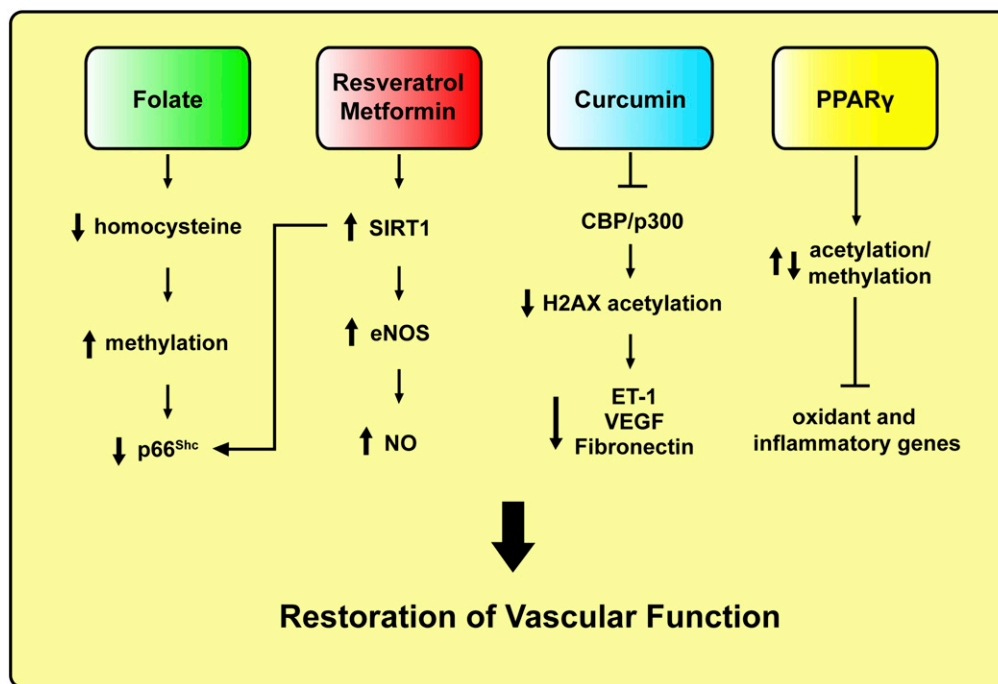
FIG. 2. Mechanism-based pharmacological approaches to revert vascular hyperglycemic memory in subjects with diabetes. ET-1, endothelin-1; H2, histone 2; VEGF, vascular endothelial growth factor.

one can postulate that metformin may contribute to reverse vascular hyperglycemic damage in patients with diabetes (Fig. 2). However, the enthusiasm of this pharmacological approach has to deal with the controversial results of the UKPDS (10) and Action to Control Cardiovascular Risk in Diabetes (ACCORD) (6) trials, where metformin was associated with either reduced or increased cardiovascular events, respectively. However, it is important to underline that despite the fact that 94.7% of patients in the intensive arm of the ACCORD trial were on metformin therapy, the drug was always given in association with other classes of glucose-lowering agents, namely, secretagogues (86.6%), thiazolidinediones (91.7%), and insulin (77.3%). Hence, it is not possible to derive any metformin-related increase in cardiovascular risk. Moreover, metformin by itself does not increase the risk of hypoglycemia, and the recent position statement of the American Diabetes Association and the European Association for the Study of Diabetes indicates metformin as the first-choice treatment for the management of hyperglycemia in type 2 diabetes (58).

Inhibitors of histone acetyltransferases have also shown to revert abnormalities linked to overexpression of prooxidant and inflammatory genes. The dietary compound curcumin, an inhibitor of the histone acetyltransferase CBP/p300, has recently been reported to prevent hyperglycemia-induced endothelial dysfunction and cardiac hypertrophy, indicating that the pharmacological removal of histone acetylation might be a promising therapeutic tool (59,60). Specifically, removal of CBP/p300-mediated histone 2XA acetylation blunts hyperglycemia-induced transcription of endothelin-1, vascular endothelial growth factor, and fibronectin, thus restoring endothelial homeostasis (59) (Fig. 2). Similarly, inhibition of the acetyltransferase GCN5 prevents angiotensin II–mediated downregulation of catalase (61), upregulation of NADPH subunit Nox2 (62), and hyperglycemia-induced p66[Shc]

overexpression (29). Moreover, CBP/p300 and GCN5 are important cell cycle regulators playing a critical role in cancer development. Phase II and III studies are currently investigating the efficacy of curcumin in this setting (63). Another interesting approach linking epigenetic modifications to diabetic vascular dysfunction is represented by the agonists of peroxisome proliferator–activated receptors (PPARs). PPARs are crucial in metabolism and adipogenesis. PPARγ ligands such as thiazolidinediones exert insulin-sensitizing and anti-inflammatory effects, primarily through action on adipocytes. Recent studies have identified a number of PPARγ-interacting partners, many of which are known epigenetic regulators, including enzymes for histone acetylation/deacetylation and histone methylation/demethylation (Fig. 2). However, their functional roles in the PPARγ transcriptional pathway are not well defined. Thanks to the advances in ChIP-based and deep sequencing technology, epigenomic mechanisms and therapeutic potentials of this nuclear receptor pathway are emerging. Epigenetic reprogramming of oxidant genes may contribute to explaining the beneficial effect of PPARγ agonists on vascular function and cardiovascular outcomes in subjects with type 2 diabetes (64). Although a clear benefit of PPARγ agonists can be postulated, cardiovascular safety of the different compounds is controversial (58). Pioglitazone may modestly reduce cardiovascular events but may also increase the risk of bladder cancer. Rosiglitazone has been shown to increase the risk of myocardial infarction and has been withdrawn in Europe and restricted in the U.S. (64). Fibrates improve cardiovascular outcomes only in diabetic patients with metabolic syndrome and dyslipidemia (58,64). Finally, the cardiovascular safety of the new pan agonist aleglitazar, currently in phase II trials, remains to be determined. The critical question of why PPARγ agonists seem to improve cardiovascular risk factors without significantly improving cardiovascular outcomes requires further investigation.

An important question to answer before the implementation of mechanism-based therapeutic strategies is whether similar pathways of hyperglycemic memory are activated in micro- and macrovasculature. Clinical observations suggest that an early intensive glycemic control may reduce first microvascular and then macrovascular complications with a timely order, suggesting microvascular disease as a prerequisite for macrovascular complications in patients with diabetes (65). Moreover, in the Atherosclerosis Risk in Communities (ARIC) study microcirculatory changes significantly correlated with macrovascular atherosclerosis and the occurrence of cardiovascular events, particularly in women (66). These findings are supported by the notion that histopathological changes of the retina and coronary arteries are similar in patients with arterial hypertension (65). Similarly, micro-angiopathic processes within the vessel wall of conduit arteries resemble that of retinal changes. These observations imply a close interaction between micro- and macrovasculature and suggest the possibility that different vascular beds may share similar molecular pathways (67). In this Perspective, metformin has shown similar benefits on micro- and macrovascular complications in the UKPDS trial (10). However, further research is needed to address these important aspects.

CONCLUSIONS

Hyperglycemic memory may explain why vascular diabetes complications progress despite intensive glycemic control, and the understanding of its mechanisms represents a real challenge to reverse persistent vascular damage and, eventually, improve cardiovascular outcome in diabetes. Additional investigations are needed to unmask the epigenetic regulation of specific genes altered by hyperglycemia. Moreover, the role of hyperglycemia in type 2 diabetes as a determinant of persistent vascular dysfunction remains to be clarified. Indeed, other important factors, namely, insulin resistance, participate in eNOS deregulation and endothelial dysfunction in this setting. Accordingly, the expression of SIRT1 and p66[Shc] is significantly affected in peripheral blood mononuclear cells of insulin-resistant individuals with type 2 diabetes (30,68). Hence, the removal of epigenetic tags resulting from SIRT1, GCN5, and Set7/9 deregulation may not be that unrealistic and may be a promising option to dampen oxidative stress and vascular inflammation and thus prevent cardiovascular complications in people with diabetes.

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