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Original Contribution

Increase in acid sphingomyelinase level in human retinal endothelial cells and CD34$^+$ circulating angiogenic cells isolated from diabetic individuals is associated with dysfunctional retinal vasculature and vascular repair process in diabetes

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BACKGROUND: Diabetic retinopathy is a microvascular disease that results from retinal vascular degeneration and defective repair due to diabetes-induced endothelial progenitor dysfunction.

OBJECTIVE: Understanding key molecular factors involved in vascular degeneration and repair is paramount for developing effective diabetic retinopathy treatment strategies. We propose that diabetes-induced activation of acid sphingomyelinase (ASM) plays essential role in retinal endothelial and CD34$^+$ circulating angiogenic cell (CAC) dysfunction in diabetes.

METHODS: Human retinal endothelial cells (HRECs) isolated from control and diabetic donor tissue and human CD34$^+$ CACs from control and diabetic patients were used in this study. ASM messenger RNA and protein expression were assessed by quantitative polymerase chain reaction and enzyme-linked immunosorbent assay, respectively. To evaluate the effect of diabetes-induced ASM on HRECs and CD34$^+$ CACs function, tube formation, CAC incorporation into endothelial tubes, and diurnal release of CD34$^+$ CACs in diabetic individuals were determined.

RESULTS: ASM expression level was significantly increased in HRECs isolated from diabetic compared with control donor tissue, as well as CD34$^+$ CACs and plasma of diabetic patients. A significant decrease in tube area was observed in HRECs from diabetic donors compared with control HRECs. The tube formation deficiency was associated with increased expression of ASM in diabetic HRECs. Moreover, diabetic CD34$^+$ CACs with high ASM showed defective incorporation into endothelial tubes. Diurnal release of CD34$^+$ CACs was disrupted with the rhythmicity lost in diabetic patients.

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CONCLUSION: Collectively, these findings support that diabetes-induced ASM upregulation has a marked detrimental effect on both retinal endothelial cells and CACs.

Introduction

Diabetic retinopathy (DR) is a microvascular disease that results from diabetes-induced retinal damage that is further exacerbated by bone marrow dysfunction. Bone marrow dysfunction leads to decreased release of cells into the circulation and changes in hematopoiesis resulting in increased circulating proinflammatory monocytes and diminished repair due to defective progenitor cells. Although DR influences all retinal cells, clinical manifestations of DR are mainly due to changes in retinal vessels, where early histologic alterations include pericyte loss, thickening of basement membrane, capillary occlusion, and endothelial cell degeneration.[1,2] These are followed by break down of blood retinal barrier and leaky vasculature leading to hemorrhages, hard exudates, and retinal edema; structural changes involving the vascular wall leading to microaneurysms; and finally neovascularization, vitreous hemorrhage, and fibrous tissue formation.[3] Impaired vision due to macular edema or vision loss due to neovascularization-induced vitreous hemorrhage or tractional retinal detachment usually takes place in the later stages of the disease.

Circulating angiogenic cells (CACs), a population of vascular progenitors hematopoietic stem cells (HSCs),[4] are considered as key regulators for healthy maintenance of retinal vasculature. Diabetic metabolic abnormalities lead to defective vascular maintenance due, in part, to failed attempts by dysfunctional CACs to repair damaged endothelium.

HSCs isolated from bone marrow or CACs from peripheral blood of control (healthy) animals have been shown to repair ischemic damage and aid in reperfusion of ischemic tissues.[4–6] Several studies have shown an association between DR risk and both reduced number[7–10] and function of CACs.[11–16]

Several key hyperglycemia- and dyslipidemia-activated pathways leading to retinal endothelial cell and CAC dysfunction have been identified. Prominent among these are pathways that promote an increase of proinflammatory cytokines, proinflammatory lipids, and proangiogenic factors.[17–26] We have previously demonstrated activation of the central enzyme of sphingolipid metabolism, acid sphingomyelinase (ASM), as a key metabolic abnormality in diabetic retinal vasculature and CACs. ASM hydrolyzes sphingomyelin into proinflammatory and proapoptotic ceramide. Activation of ASM plays an important role in signal transduction in response to various stimuli including interleukin-1β[27,28] and tumor necrosis factor-α.[29] Endothelial cells represent a major source of ASM.[30–33] Inhibition of ASM exhibits protective effect in diabetes preventing diabetes-induced retinal inflammation and vascular degeneration.[15,33,34]

Previously, we have identified key defects in circadian regulation of CACs. We showed that bone marrow denervation results in loss of circadian release of vascular reparative cells from the bone marrow and generation of increased numbers of proinflammatory cells. Using a rat model of type II diabetes, we showed that the decrease in CACs release from diabetic bone marrow is caused by bone marrow neuropathy and that these changes precede the development of DR. We observed a marked reduction in clock gene expression in the retina and in CACs. Denervation of the bone marrow resulted in progenitors being "trapped" within the bone marrow and in loss of the circadian release of these cells into the circulation. This reduction in the circadian peak of CAC release into the circulation led to diminished reparative capacity and resulted in development of acellular retinal capillaries.[7] We also showed that Per2 mutant mice recapitulate key aspects of diabetes without the associated metabolic abnormalities. In Per2 mutant mice, we observed a 3-fold decrease in proliferation and 50% reduction in nitric oxide levels in CACs. Tyrosine hydroxylase–positive nerve processes and neurofilament-200 staining were reduced in Per2 mutant mice (suggestive of diabetic neuropathy) and increased acellular capillaries were identified.[35] We also showed that as CD34+ CACs acquired differentiation markers (toward the endothelial lineage), robust oscillations of clock genes are observed.[36]

It is well accepted in diabetic complications field that cells isolated from diabetic tissue keep diabetic phenotype for several passages even when cultured in normal glucose. This is due to "metabolic memory," or "legacy effect" for vascular disease in diabetes—the prolonged benefits of good glycemic control, as well as the prolonged harm of poor control in diabetic patients.[11,37–39] In this study, we used HREC cells isolated from control and diabetic donor tissue as a model.

In the present study, we have focused exclusively on human CACs. We asked if the defect in circadian release observed in rodents with diabetes occurred in humans. We examined the effect of diabetes-induced ASM activity on the function of human CACs and retinal endothelial cells comparing the angiogenic ability of control (low ASM) and diabetic (high ASM) HRECs to form tube-like structures in vitro and determining the capacity of control (with low ASM) and diabetic (with high ASM) CACs to support endothelial tube formation.