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14. ABSTRACT

Diabetes mellitus is a devastating metabolic disease characterized by hyperglycemia that can occur through distinct mechanisms, such as islet beta-cell destruction, beta-cell failure, and insulin resistance in peripheral tissues. The increasing prevalence of diabetes also affects the military personnel. Several genes associated with diabetes have been identified in genome wide association studies, but their genetic validation as causative factors remains to be demonstrated experimentally. In this grant proposal, we focused on one of these genes, TGIF, whose conditional overexpression in the pancreatic tissue led to severe hyperglycemia and diabetes. We proposed mechanistic experiments to investigate how TGIF1 induces diabetes. Our preliminary findings strongly suggest that TGIF overexpression in the pancreatic tissue induced beta-cells, providing a mechanism by which TGIF drives diabetes.

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Progress Report Atfi (DOD award# W81XWH-17-1-0116)

Due to the transfer of my lab to Virginia Commonwealth University, we interrupted all the experiments described in our grant application, pending approval of our subcontract with the University of Mississippi Medical Center by the Department of Defense. We provide below a summary of our research project as well as a brief description of the results of the experiments performed before moving from the University of Mississippi Medical Center to Virginia Commonwealth University.

Introduction

Diabetes is a complex disease caused by abnormal expression of multiple genes that govern critical aspects of pancreatic development and homeostasis. Several potential genes associated with diabetes have been identified by integrative genomic approaches, but their validation as causative factors remains to be established. In this proposal, we intended to focus our efforts on one of these candidate diabetes genes, *TGIF*, which encodes for a transcriptional repressor known to govern fundamental biological processes crucial for proper body development and maintenance of organ homeostasis throughout life. Our preliminary data showed that enforced expression of TGIF in the pancreatic epithelium in mice resulted in high hyperglycemia, reminiscent of diabetes. Based on this novel observation, we hypothesized that TGIF overexpression might affect insulin production by islets β-cells, thereby culminating in insulin insufficiency and attendant hyperglycemia and diabetes. To test this overreaching hypothesis, we proposed to conduct genetic experiments using mice harboring either conditional overexpression (*Tgif*+) or conditional knockout (*Tgif.KO*) of *Tgif* in the pancreatic tissue. We designed several approaches to conduct full analyses of the diabetic phenotype of *Tgif*+ mice, including pancreas histology, blood glucose level, serum insulin levels, glucose tolerance, insulin tolerance and β-cell mass, proliferation, apoptosis and dedifferentiation. To corroborate the role of endogenous TGIF in driving β-cell dysfunction, we proposed to challenge *Tgif.KO* mice with high fat diet and carry out the same experiments as described earlier for *Tgif*+ mice. To delineate the molecular mechanisms by which TGIF1 affects β-cell homeostasis and insulin production, we designed molecular and biochemical studies aimed at elucidating whether TGIF functions to repress transcription of

the *Pdx1* gene (pancreatic duodenal homeobox-1), which encodes for a master transcription factor that directly regulate synthesis and production of insulin by islet β-cells.

Accomplishments

To test our overarching hypothesis, we proposed to develop the following specific aims:

Specific Aim 1: **Achieve a comprehensive characterization of TGIF's ability to promote diabetes**, with particular emphasis on blood glucose and serum insulin levels, glucose tolerance, and β-cell function and mass in mice bearing either conditional overexpression or conditional knockout of *Tgif* in the pancreatic tissue.

Specific Aim 2: **Explore the molecular mechanisms by which TGIF affects β-cell function**, focusing on its ability to repress expression of the *Pdx1* gene, which encodes the master transcription factor in β-cells.

These specific aims remained unchanged. Overall, we performed many key experiments described in our original grant application, and the data clearly showed that overexpression of TGIF in the pancreatic tissue plays a major role in diabetes.

Summary of results obtained

To investigate the role of TGIF in pancreas development, we conducted genetic studies using mice with pancreas-specific overexpression of *Tgif* (*Tgif*+). Accordingly, we crossed mice bearing a Cre-activable *TGIF* transgene, *LSL-Tgif,* with *Pdx1.Cre* mice, which express Cre recombinase in all pancreatic progenitor cells. *Tgif*+ mice were born at the expected Mendelian ratio, showed no evidence of any gross anatomic or physiological abnormalities, and had normal weight at birth, indicating that TGIF overexpression in the pancreas glandular does not affect early development. Intriguingly, measuring blood glucose of 4-week-old *Tgif*+ animals revealed severe hyperglycemia, which was associated with low circulating insulin levels. At necropsy, the pancreatic tissues of *Tgif*+ mice displayed almost normal appearance, though there was a slight but significant decrease in their weight. In efforts to investigate the mechanisms behind this phenotype, we performed several experiments to

analyze pancreas histology using *Tgif*+ and control mice of 4 to 8 weeks of age. As assessed by hematoxylin and eosin (H&E) staining, we detected a complete disorganization in the architecture of the islets, often resulting in islets void of β-cells in the central area. Immunohistochemistry experiments using anti-cleaved caspase 3 detected a massive increase in apoptosis within the islets, providing a potential mechanism by which TGIF overexpression leads to diabetes. Collectively, these findings strongly suggest that TGIF overexpression drives diabetes by affecting islet β-cells survival.

We will initiate the remaining experiments within the next few days following final approval by the Sponsored Programs of both Virginia Commonwealth University and the University of Mississippi Medical Center.