The Expression of GPR109A, NF-κB and IL-1β in Peripheral Blood Leukocytes from Patients with Type 2 Diabetes

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Abstract. This study was designed to explore the association between the G protein-coupled receptor 109A (GPR109A) expression in peripheral blood leukocytes (PBLs) and type 2 diabetes (T2DM) and to discuss the regulation of inflammatory factors by GPR109A signaling. GPR109A signaling has been confirmed to be associated with homeostasis of glucose/lipid metabolism, but the role of signaling in T2DM is still poorly understood. Peripheral blood samples and biochemical data were collected from healthy individuals (normal controls) and T2DM patients. Immunocytochemical staining was used to detect the expression of GPR109A in PBLs. Reverse transcription polymerase chain reaction (RT-PCR) was used to measure mRNA levels of GPR109A, NF-κB, and IL-1β in PBLs. Immunocytochemical staining showed that the GPR109A protein is localized in the nucleus and cytoplasm of granulocytes, monocytes, and lymphocytes. RT-PCR showed that mRNA levels of GPR109A, NF-κB, and IL-1β were higher in the T2DM group than in the control group (P<0.05). Correlation analysis showed a positive correlation both between GPR109A/NF-κB (r=0.376, P<0.05), and GPR109A/IL-1β (r=0.501, P<0.05) and between GPR109A and fasting plasma glucose (FPG) (r=0.179, P<0.05) and NF-κB/FPG (r=0.358, P<0.05). Our results suggest that GPR109A signaling is associated with T2DM, playing a role in regulation of the inflammatory cytokines.

Key words: GPR109A, NF-κB, IL-1β, Pancreatic islet β-cell, Type 2 diabetes, peripheral blood leukocytes

Introduction

With the improvement of living standards, the rise in dyslipidemia and the incidence of diabetes are annually increasing worldwide. As both are involved in metabolic syndrome, the complicated relationship between diabetes and dyslipidemia has become a focus of investigation in recent years. While niacin, a pharmacological agent, has been used to treat dyslipidemia for more than 50 years, the recent discovery of the niacin receptor provides a novel target for therapeutic agents. The niacin receptor, a seven-transmembrane G protein-coupled receptor of the Gi family, was formerly identified as the G protein-coupled receptor 109A (GPR109A, also HM74A or HCA2) in humans and as a protein up-regulated in macrophages by INF-γ(PUMA-G) in murine [1].

As the molecular mechanism of this receptor has been more understood, evidence indicates that it may be a intersection between glycometabolism and lipid metabolism. GPR109A binds to nicotinic acid and is responsible for the antilipolytic and triglyceride-lowering effects of nicotinic acid [2,3,4]. Non immune cells express the niacin receptor, suggesting that GPR109A may also play a role in the regulation of metabolic disorders, inflammatory diseases, or other diseases that lie beyond lipoprotein modulation. As Martin et al. showed, GPR109A is expressed in retinal pigment epithelium (RPE). cAMP assays demonstrate that RPE GPR109A is functional, suggesting that GPR109A may have biologic importance in diabetic retinopathy [5]. Retinal GPR109A expression increases in human diabetes and in a model [6]. Activation of GPR109A in cultured RPE cells suppresses TNF-α-induced production of proinflammatory cytokines IL-6 and Ccl2. In contrast, GPR109A ligands fail to suppress these effects in primary RPE cells from GPR109a/-mice, confirming that the
observed anti-inflammatory effects were mediated specifically by GPR109A. Since T2DM can be considered a systemic chronic inflammatory disease, these results suggest that increased expression of GPR109A in diabetic RPE cells [6] could inhibit the inflammatory response in an attempt to protect the retina. Decreasing serum levels of adiponectin has been correlated with a risk for development of type 2 diabetes, impairing glucose tolerance [7], but niacin can stimulate adiponectin secretion by activating GPR109A [8]. More intriguingly, high levels of PUMA-G transcripts and protein in the pancreas are detected in all β-cells, and approximately 40% of pancreatic islet α-cells up-regulate PUMA-G expression with age. Furthermore, static incubation of islets with niacin leads to an approximately 30% reduction of glucose-stimulated insulin secretion (GSIS) [9]. With increasing evidence that GPR109A expression in diabetic RPE cells is higher and plays a role in the regulation of insulin secretion, we hypothesise that GPR109A signaling pathway may be relevant to the development of diabetes.

Peripheral blood mononuclear cells isolated from patients with diabetic nephropathy had the oxidative-stress sensitive transcription factor NF-κB increased in activation [10]. Furthermore, neutrophil and monocyte adherence was markedly impaired in diabetes mellitus [11]. T2DM is a systemic chronic inflammatory disease, exhibiting a decline in the function of β-cells and thus reducing insulin secretion and increasing blood sugar. While leukocytes, as inflammatory cells, reveal a discrepancy of metabolism in T2DM, thus, the peripheral blood leukocytes (PBLs) function decline, may affecting the expression of GPR109A. To investigate whether a relationship exists between GPR109A expression in the PBLs and T2DM, we examined and compared the expression levels of GPR109A in healthy individuals (normal controls) and T2DM patients in this study. Then, we investigated the relationship between the expression of GPR109A and that of transcription factor NF-κB and proinflammatory cytokine IL-1β, in an attempt to explore the association between GPR109A expression in the PBLs and T2DM.

Materials and Methods

Research subject. 152 T2DM patients [72 males and 80 females, 43-76 years of age (59.29±8.47 years)], who were hospitalized in the First Affiliated Hospital of Shantou University Medical College from September 2012 to April 2013, were involved in this study. All patients met the 1999 WHO diagnostic criteria for diabetes. 93 normal healthy individuals [49 males and 44 females, 40-76 years of age (52.23±9.42 years)], who came to the First Affiliated Hospital of Shantou University Medical College for a physical examination from September 2012 to April 2013, were designated as the control group. Every patient and control subject signed an informed consent form. The study protocol was approved by the Independent Ethics Committee of the First Affiliated Hospital of Shantou University Medical College and was performed in accordance with the Declaration of Helsinki.

Methods. Peripheral blood and biochemical data were collected from every patient and control subjects.

PBL collection. Fresh anticoagulated peripheral blood (3 mL) was collected from each subject. Red blood cells (RBC) were dissolved with RBC lysis solution (9.34g/L) for 30 min. Then, the solution was centrifuged for 5 min, and the supernatant discarded, leaving the PBLs population. Total RNA was extracted and used for RT-PCR using standard methods.

Immunocytochemical staining. Immunocytochemical staining was performed on peripheral blood smears to detect GPR109A protein expression. All procedures were carried out at room temperature. Blood smears were fixed with 4% paraformaldehyde for 20 min and then with 0.3% H2O2 incubate for 10 min. Samples were then blocked with 2% Bovine Serum Albumin (BSA), and incubated with primary antibody against GPR109A (1:50 diluted in PBS; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) for 30 min. Then, the solution was centrifuged for 5 min, and the supernatant discarded, living the PBLs population. Total RNA was extracted and used for RT-PCR using standard methods.
Reverse transcription polymerase chain reaction (RT-PCR). RT-PCR was used to detect the relative expression levels of GPR109A, NF-κB, and IL-1β mRNA in PBLs. Total RNA was isolated from PBLs using the RNA simple Total RNA Kit (Tiangen Biotech Beijing, China). First-strand cDNAs were generated by EasyScript First-stand cDNA Synthesis SuperMix Kit (TransGen Biotech, Beijing, China). Primer sequences (BGI, Beijing, China) are shown in **Table 1**. After cDNA synthesis, PCR was performed using the EasyTaq PCR SuperMix kit (TransGen Biotech, Beijing, China) for 35 cycles of denaturing at 94°C for 30 s, annealing at 62 °C for 30 s, extension at 72°C for 1 min, and a final 10 min extension at 72°C. PCR products were subjected to 1% agarose gel electrophoresis and visualized with ethidium bromide. Relative expression was quantified densitometrically using a Gel Image Ver 3.74 System (Tianon, Shanghai, China).

**Statistical analysis.** Statistical analysis was performed with IBM SPSS Statistics 19 software. All values were expressed as the mean ± standard deviation. The
statistical analysis was done by independent-samples T-test, and comparisons were made by the χ²-test. Linear correlation was used for correlative analysis. Differences with P<0.05 were considered statistically significant.

Results

Clinical characteristics of the two groups. Fasting plasma glucose (FPG), triglyceride (TG), and low density lipoprotein (LDL) levels were significantly higher in the T2DM group than in the control group (P<0.05). Total cholesterol (TC) and high density lipoprotein (HDL) levels were not significantly different in both groups (P>0.05) (Table 2). PBLs (8.85±4.52)*10⁹/L were 33% higher in the T2DM group than in the control group (6.65±1.53)*10⁹/L (P<0.05) (Table 2).

Immunocytochemical staining. The results showed that the GPR109A protein was localized in the nucleus and cytoplasm of granulocytes, monocytes, and lymphocytes (Figure 1).

RT-PCR results. The mRNA expression levels of GPR109A, NF-κB, and IL-1β were significantly higher in the T2DM group than in the control group (P<0.05) (Figure 2).

Correlation analysis between GPR109A, NF-κB and IL-1β, and blood glucose/lipid. Correlation analysis showed that a positive correlation between GPR109A and NF-κB (r=0.376, P<0.05) and between GPR109A/IL-1β(r=0.501, P<0.05) and NF-κB/IL-1β(r=0.154, P<0.05) (Table 3). There was also a positive correlation between GPR109A and fasting plasma glucose (FPG) (r=0.179, P<0.05, and NF-κB/FPG (r=0.358, P<0.05) (Table 4).

Discussion

Clinical characteristics of the two groups showed that FPG, TG, LDL levels were all higher in the T2DM group than in the normal control group. This result implies that diabetes mellitus is always accompanied by dyslipidemia. Niacin, by reducing cAMP levels, inhibits the activity of lipase and prevents the release of free fatty acids from fat stores. It has been used for the treatment of dyslipidemia for more than 50 years. Indeed, GPR109A has been confirmed to bind to niacin and is responsible for the antilipolytic and triglyceride-lowering effects of niacin [2,3,4].

Due to its expression in adipocytes, isletβ-cells, and RPE cells, GPR109A signaling has generated increasing interest. This signaling may enable cross-talk between glycometabolism and lipid metabolism, and even between metabolic disorders and inflammatory. In our study, RT-PCR showed that mRNA levels of GPR109A, NF-κB, and IL-1β were higher in the T2DM group than in the control group. This is in accordance with previous observations that, under high glucose concentration, expression of the niacin receptor PUMG-G is
up-regulated [6]. Animal experiments show that static incubation of islets with niacin lead to significant reduction in levels of GSIS [9]. Therefore, it is conceivable that the niacin receptor may play a role in protecting islet \( \beta \) cells in cases of sustained high glucose concentration. Correlation analysis of this study showed that a significant correlation between GPR109A and fasting blood-glucose (FBG), indicating that hyperglycemia may lead to the up-expression of GPR109A, serving as a negative feedback mechanism to inhibit insulin secretion and thereby delaying the beta cell failure. However, our results demonstrated that there is no significant correlation between GPR109A and blood-lipid. This is consistent with the viewpoint that the GPR109A signaling pathway remains a complicated mechanism, whose regulation of glycometabolism and lipid metabolism has not yet been identified.

NF-\( \kappa \)B, a key mediator of inflammation that is detected in almost all mammalian cells, is activated via toll-like receptors (TLRs), resulting in increasing cytokine and chemokine production [12]. Activation of NF-\( \kappa \)B and release of its subunits is related to many diseases such as cancer, inflammation, and autoimmune diseases. Indeed, previous reports have indicated that type 2 diabetic subjects have decreased IkB muscle content, suggesting enhanced IkB/NFkB signaling [13]. When cytokines, including TNF\( \alpha \) and IL-6, activate the IkB/NFkB pathway, a positive feedback loop is formed to induce the inflammatory reaction, leading to insulin resistance. Intriguingly, a study on GPR109A function as a tumor suppressor in the colon showed that the receptor plays a critical role in the prevention of colon cancer and inflammatory bowel disease by suppressing NF-\( \kappa \)B activation [14]. IL-1\( \beta \), an inflammatory cytokine that is a crucial downstream target of NF-\( \kappa \)B, is involved in the destruction of islet A cells during the development of type 1 diabetes and also promotes the apoptosis of beta cells. Studies have showed that the blockade of interleukin-1 with anakinra improved glycemia and \( \beta \)cell secretory function and reduced markers of systemic inflammation [15]. Our study showed that NF-\( \kappa \)B and IL-1\( \beta \)expression is significantly higher in the T2DM group than in healthy individuals. The positive correlation between NF-\( \kappa \)B and FPG (\( r=0.358, P<0.05 \)) suggested that activation of NF-\( \kappa \)B is closely related to FPG. Furthermore, our finding showed a positive correlation between GPR109A/NF-\( \kappa \)B (\( r=0.376, P<0.01 \)) and GPR109A/IL-1\( \beta \) (\( r=0.501, P<0.01 \)), demonstrating that GPR109A may be capable of directly or indirectly influencing the expression of NF-\( \kappa \)B and IL-1\( \beta \) and thus could play a regulatory role of inflammatory environment in diabetes. However, further exploration and research are needed to identify the mechanisms.

Neutrophil and monocyte adherence is markedly impaired in diabetes mellitus [11]. More recently, Antonio et al. showed that mitochondrial function in leukocytes is impaired in type 2 diabetic patients [16]. As suggested by our study, hyperglycemia may be one factor which impairs leukocyte function and further influences cytokine expression in leukocytes via up-regulation of GPR109A, NF-\( \kappa \)B and IL-1\( \beta \). Furthermore, as our data shows, there is a significant increase in leukocytes in the T2DM group, as compared with the healthy individuals. This corresponds with the evidence that the proliferation,
senescence, and apoptosis of leukocytes, as well as the inflammatory cells, are likely to be associated with T2DM, a systemic chronic inflammatory disease. GPR109A, NF-κB, and IL-1β expression in leukocytes is also likely to be affected by the proliferation, aging, and apoptosis of leukocytes. This leads us to believe that GPR109A, NF-κB, and IL-1β participate in the development of T2DM, although this needs further studies to be confirmed.

In summary, our study suggests that GPR109A signaling may play a role in the regulation of inflammatory cytokines and be associated with T2DM.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Project No. 81370925, 81373745 and 81172894) and the National Natural Science Foundation of Guangdong Province (Project No. 2008B030301369 and 2012B021800254).

References


