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Anti-apoptotic activity of human matrix metalloproteinase-2 attenuates diabetes mellitus

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ABSTRACT

Background: Chronic progression of diabetes is associated with decreased pancreatic islet mass due to apoptosis of β-cells. Patients with diabetes have increased circulating matrix metalloproteinase-2 (MMP2); however, the physiological significance has remained elusive. This study tested the hypothesis that MMP2 inhibits cell apoptosis, including islet β-cells.
Methods: Samples from diabetic patients and newly developed transgenic mice overexpressing human MMP2 (hMMP2) were harnessed, and diabetes was induced with streptozotocin.
Results: Circulating hMMP2 was significantly increased in diabetic patients compared to controls and significantly correlated with the serum C-peptide levels. The diabetic hMMP2 transgenic mice showed significant improvements in glycemia, glucose tolerance and insulin secretion compared to diabetic wild type mice. Importantly, the increased hMMP2 levels in mice correlated with significant reduction in islet β-cell apoptosis compared to wild-type counterparts, and an inhibitor of hMMP2 reversed this mitigating activity against diabetes. The increased activation of Akt and BAD induced by hMMP2 in β-cells compared to controls, links this signaling pathway to the anti-apoptotic activity of hMMP2, a property that was reversible by both an hMMP2 inhibitor and antibody against integrin-β3.
Conclusion: Overall, this study demonstrates that increased expression of hMMP2 may attenuate the severity of diabetes by protecting islet β-cells from apoptosis through an integrin-mediated activation of the Akt/BAD pathway.

1. Introduction

Diabetes mellitus (DM) is a major global health problem with >300 million people living with DM worldwide [1,2]. DM is the fourth highest cause of death and one of the most important risk factors for disabling diseases including retinopathy, peripheral ischemic angiopathy, ischemic heart disease and nephropathy with renal failure [1,3]. Palliative, but no curative, therapy is currently available for type 1 and type 2 clinical forms of DM [4]. The etiology has been associated with genetic or autoimmune factors in type 1 DM, and with lifestyle-related insulin resistance and/or abnormal insulin secretion in type 2 DM. Histopathological studies have shown that apoptosis with reduced number of insulin-secreting pancreatic islet β-cells are common features in both types of DM [4,5]. However, the underlying regulatory mechanism of islet β-cell apoptosis is not completely understood.

Matrix metalloproteinases (MMPs) are a large group of calcium-dependent zinc-containing endopeptidases that play critical roles in wound healing, tissue repair/remodeling and morphogenesis [6]. The human MMP family comprises 24 members synthesized in a variety of cells and released as latent proenzymes (pro-MMPs). Plasmin, urokinase and other proteases can activate pro-MMPs. Apart from degrading

Abbreviations: MMP2, matrix metalloproteinase-2; TG, transgenic; STZ, streptozotocin; DOX, doxycycline; NF, normal food; FCS, fetal calf serum; HUVECs, human umbilical vein endothelial cells; NHMCs, normal human mesangial cells; EDTA, ethylenediaminetetraacetic acid; WT, wild type; SAL, saline.

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connective tissue components, the active MMPs can also cleave and release active growth factors, membrane-anchored receptors, and cell-adhesion or apoptosis-related molecules, and by this mechanism, they can indirectly regulate cell proliferation, motility, survival and angiogenesis [6–8]. MMP2, a 72-kDa type IV collagenase, is a member of this MMP family that plays an important role in several physiological and pathological processes. A recent study suggested that pro-MMP2 can also directly affect cell function by stimulating the secretion of vascular endothelial growth factor to promote angiogenesis [9]. However, to date there is no study showing effects of MMP2 on cell apoptosis.

Increased level of the proenzyme and active forms of MMP2 has been previously reported in the peripheral blood from patients with diabetes and metabolic syndrome [10–15]. In addition, clinical correlation studies suggested that high circulating MMP2 level may indicate severity of microangiopathic complications in advanced stages, but its significance in early stages of diabetes is unknown [12,14]. Previous studies have demonstrated that MMPs can interact with integrins, thereby regulating cell survival by activating Akt and that MMP2 can also modulate the Akt signaling pathway [9,16–21]. The aim of the present study was, therefore, to test the hypothesis that human MMP2 (hMMP2) can inhibit pancreatic β-cell apoptosis by interacting with integrin-β3 and thereby activating the Akt/BAD pathway, and that overexpression of hMMP2 attenuates diabetes in mice.

2. Materials and Methods

2.1. Subjects

Blood samples were made available by 22 patients with type 2 DM (mean age 55.6 ± 10.2 year-old; females 9, males 13) with different durations of diabetes, and 34 healthy volunteers (mean age 53.9 ± 7.6 year-old; females 25, males 9) to measure clinical parameters. Table 1 shows the demography and laboratory data of the subjects. Written informed consent was given by all patients and healthy subjects, and the study protocol was approved by the Ethics Committee for Clinical Investigation of Mie University (approval No 107 and 2194).

2.2. Animals

2.2.1. Generation of hMMP2 Overexpressing Transgenic (hMMP2-TG) Mice

The hMMP2-TG mouse with C57BL/6 J genetic background was generated using a full-length hMMP2 cDNA subcloned into a vector containing the CAG-promoter [cytomegalovirus enhancer + chicken β-actin promoter] and rabbit β-globin polyadenylation sites (Riken Bioresources, Tsukuba, Japan). The hMMP2 cDNA of pENTR221 vector was subcloned into the pBS-CAG-DEST vector using the Gateway technology (Invitrogen), and the sequence of the hMMP2 cDNA was confirmed by direct sequencing and by restriction analysis (Supplementary Fig. 1A,B,C). Expression of hMMP2 cDNA was driven by the chicken β-actin promoter linked to a human cytomegalovirus immediate-early enhancer, followed by the first exon and intron of chicken β-actin (Fig. 1A). The vector was digested, purified, and microinjected into fertilized eggs from C57BL/6J mice and then implanted (CLEA Japan Inc.). Transgenic founders were assessed by Southern blotting and genotyping by polymerase chain reaction (PCR).

C57BL/6 wild-type (WT) mice were used as controls. Male WT and hMMP2-TG mice 15-to-17-weeks old and weighing 20–40 g were used in the experiments and they were maintained in a specific pathogen-free environment and subjected to a 12-h light:dark cycle in the Mie University animal house. The Committee on Animal Investigation of Mie University approved the experimental protocols (Approval No 24–50) and all procedures were carried out following the institutional guidelines. All mice received humane care according to the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health. The research followed the ARRIVE Guidelines for animal investigation. Mice were randomized and researchers that measured the parameters in the samples were blinded to the treatment groups.

2.2.2. DM Mouse Model

Mice were randomized, all mice that started the experiments were accounted for, and researchers that measured parameters were blinded to treatment groups. DM was induced in WT (WT/STZ) and hMMP2-TG (hMMP2-TG/STZ) mice by intraperitoneal injection of streptozotocin (STZ; Sigma-Aldrich, St. Louis, MO) at a dose of 40 mg/kg for 5 consecutive days. Control mice were injected with the same volume of saline (SAL) solution (WT/SAL, hMMP2-TG/SAL) for 5 consecutive days. We sacrificed all mice after 4 weeks of STZ administration.

2.2.3. DM Evaluation

Fasting blood glucose levels were measured once a week for 4 weeks. On the 4th week after STZ or saline injection, an intraperitoneal glucose tolerance test (IPGTT) was performed after 16 h fasting by intraperitoneal glucose injection (1 g/kg mouse body weight). One day later, a glucose stimulated insulin secretion test was performed after 16 h fasting by intraperitoneal glucose injection (3 g/kg mouse body weight). Tail vein blood was drawn for measurement of glucose and insulin levels. After completion of the insulin secretion test, mice were sacrificed by pentobarbital overdose and pancreatic tissues collected for subsequent evaluation.

2.2.4. Histopathological Evaluation

After sacrifice of mice by anesthesia overdose, the pancreas was resected, dehydrated, embedded in paraffin, and then 3-μm-thick sections were prepared. Immunostaining of insulin and glucagon was performed at Biopathology Institute Corporation by using antibodies from Dako Corporation (Carpinteria, CA). All visible islets (WT/SAL [n = 4], hMMP2-TG/SAL [n = 3], WT/STZ [n = 6], hMMP2-TG/STZ [n = 6]) in

Table 1
Demography and laboratory data of healthy and diabetic subjects.

	All subjects			Males			Females		
	Healthy controls	Type 2 Diabetes	p values	Healthy controls	Type 2 Diabetes	P values	Healthy Controls	Type 2 Diabetes	p values
Number	34	22		9	13		25	9	
Duration of DM (years)		15.6 ± 12.4			18.2 ± 11.3			11.8 ± 13.7	
Age (year-old)	53.9 ± 7.6	55.6 ± 10.2	0.47	52.3 ± 8.5	56.5 ± 12.1	0.38	54.5 ± 7.3	54.3 ± 7.3	0.96
BMI	23.7 ± 3.7	24.5 ± 3.6	0.45	23.0 ± 3.3	24.8 ± 3.2	0.22	24.0 ± 3.9	24.0 ± 4.2	0.96
Serum hemoglobin A1c (%)	5.7 ± 0.6	9.4 ± 2.0	<0.0001	5.5 ± 0.4	8.9 ± 1.5	<0.0001	5.8 ± 0.6	10.1 ± 2.5	<0.0001
Fasting blood glucose (mg/dL)	96.5 ± 15.6	201.2 ± 82.1	<0.0001	91.4 ± 9.7	196.8 ± 70.5	0.0003	98.3 ± 17.0	207.4 ± 100.9	<0.0001
Serum total cholesterol (mg/dL)	216.7 ± 30.9	194.2 ± 50.0	0.04	203.7 ± 30.6	180.9 ± 40.2	0.17	221.4 ± 30.2	213.4 ± 58.5	0.61
Serum triglycerides (mg/dL)	121.4 ± 61.8	234.2 ± 242.1	0.01	145.6 ± 84.8	256.8 ± 301.1	0.30	112.7 ± 50.6	201.6 ± 126.1	0.055
Serum HDL lipoproteins (mg/dL)	59.4 ± 14.6	41.8 ± 12.5	<0.0001	54.9 ± 8.8	39.9 ± 11.4	0.0035	61.1 ± 16.0	44.6 ± 14.2	0.01
Plasma MMP-2 (ng/mL)	54.0 ± 27.5	79.8 ± 31.7	0.002	54.6 ± 18.3	79.3 ± 27.1	0.03	53.7 ± 30.5	80.5 ± 39.3	0.044
Serum C-peptide (ng/ml)		2.2 ± 2.1			1.7 ± 1.0			3.0 ± 3.0	

BMI, body mass index; HDL, high-density lipoprotein; MMP-2, matrix metalloproteinase-2.
Data are the mean ± S.D.