METABOLIC, ENDOCRINE, AND GENITOURINARY PATHOBIOLOGY

Matrix Metalloproteinase-9 Is Essential for Physiological Beta Cell Function and Islet Vascularization in Adult Mice

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Accepted for publication
December 22, 2014.

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The availability of paracrine factors in the islets of Langerhans, and the constitution of the beta cell basement membrane can both be affected by proteolytic enzymes. This study aimed to investigate the effects of the extracellular matrix-degrading enzyme gelatinase B/matrix metalloproteinase-9 (Mmp-9) on islet function in mice. Islet function of *Mmp9*-deficient (*Mmp9*^{−/−}) mice and their wild-type littermates was evaluated both *in vivo* and *in vitro*. The pancreata of *Mmp9*^{−/−} mice did not differ from wild type in islet mass or distribution. However, *Mmp9*^{−/−} mice had an impaired response to a glucose load *in vivo*, with lower serum insulin levels. The glucose-stimulated insulin secretion was reduced also *in vitro* in isolated *Mmp9*^{−/−} islets. The vascular density of *Mmp9*^{−/−} islets was lower, and the capillaries had fewer fenestrations, whereas the islet blood flow was threefold higher. These alterations could partly be explained by compensatory changes in the expression of matrix-related proteins. This in-depth investigation of the effects of the loss of MMP-9 function on pancreatic islets uncovers a deteriorated beta cell function that is primarily due to a shift in the beta cell phenotype, but also due to islet vascular aberrations. This likely reflects the importance of a normal islet matrix turnover exerted by MMP-9, and concomitant release of paracrine factors sequestered on the matrix. (*Am J Pathol* 2015, 185: 1094–1103; <http://dx.doi.org/10.1016/j.ajpath.2014.12.009>)

The presence of matrix metalloproteinases in pancreatic islets was described more than 15 years ago,^{1,2} but their exact role for islet function has still not been determined. Some of these enzymes have been implicated as important in pancreas development and islet morphogenesis,^{3,4} whereas other reports indicate that, at least, the most studied metalloproteinases, matrix metalloproteinase-2 (MMP-2) and MMP-9 are dispensable in this aspect.⁵ Proteinases hold the potential to affect the morphology and function of pancreatic islets not only by direct effects, but preferentially by regulating the availability of different growth factors and chemokines bound to the extracellular matrix.^{6,7} Thus, the predominant proangiogenic effect of MMP-9 is mediated by the release of vascular endothelial growth factor A (VEGF-A) from macromolecules during matrix degradation, which turns VEGF-A accessible as a mitogen for endothelial cells.⁸ Indeed, MMP-9 has been implicated as a key player in the angiogenic shift during

development of islet tumors, as well as in revascularization of transplanted pancreatic islets.^{8–10}

The importance of the islet vasculature for the endocrine function of the pancreatic islets has gained considerable interest in recent years. The islets are among the best blood-perfused organs in the body,^{11,12} where each beta cell is in

Supported by the Swedish Research Council (M.P. and P.-O.C.), the Swedish Diabetes Fund (Diabetesfonden) (M.P., P.-O.C., and G.C.), the Swedish national strategic research initiative EXODIAB (Excellence of Diabetes Research in Sweden) awarded to Uppsala and Lund Universities, Diabetes Wellness Sweden (M.P. and P.-O.C.), the Ragnar Söderberg Foundation (M.P.), the Knut and Alice Wallenberg Foundation (M.P.), the Swedish Diabetes Foundation (Svenska Diabetesstiftelsen) (G.C.), the Family Ernfrors Foundation (M.P.), the Novo Nordisk Foundation (M.P. and P.-O.C.), the Fund for Scientific Research of Flanders (FWO-Vlaanderen) (G.O.), and the Geconcerteerde OnderzoeksActies (GOA 2013/014) in Belgium (G.O.).

P.-O.C. and M.P. contributed equally as senior authors.

Disclosures: None declared.

direct contact with at least one endothelial cell.¹³ Thus, the endothelial cells are constantly interacting with the endocrine cells through paracrine influence,¹⁴ and several endothelial cell–derived factors such as laminins,^{15,16} thrombospondin-1,¹⁷ and hepatocyte growth factor¹⁸ have been shown to be of importance for beta cell function, differentiation, and proliferation in the adult.

The high level of tortuous vascularization of the islets is due to the release of VEGF-A from beta cells, and mice incapable of beta cell synthesis of VEGF-A exhibit markedly decreased islet capillary density, as well as nonoptimal blood glucose control in the adult.¹⁹ The maintained release of VEGF-A from beta cells is of importance also during adulthood to preserve the high level of vascularization as well as keeping the microvascular endothelium in a highly fenestrated state.^{20,21} Genetic interference with the VEGF-A gene in beta cells in the adult mouse will not affect islet mass or insulin secretion *in vitro*, but rather will affect the islet endothelium to reduce fenestration and thereby delay insulin release.²⁰

Here, we tested the hypothesis that MMP-9 plays a critical role in beta-cell function and islet vascular homeostasis in postnatal islets. This was addressed in *Mmp9*-deficient (*Mmp9*^{−/−}) mice by both *in vivo* and *in vitro* experiments to be able to discriminate between alterations of the beta-cell phenotype and indirect effects on blood glucose homeostasis mediated by vascular changes, ie, alterations in vascular density, blood perfusion, or capillary fenestrations.

Materials and Methods

Animals

Male *Mmp9*^{−/−} mice,²² backcrossed 12 generations to C57Bl/6 and their wild-type (WT) littermates (25 to 30 g; Rega Institute, Leuven, Belgium), at 3 to 6 months of age had free access to tap water and pelleted food throughout the study. All experiments were approved by the Uppsala Regional Laboratory Animal Ethics Committee.

Glucose Tolerance Tests

Glucose [2.5 g/kg body weight (b.w.); Fresenius-Kabi, Uppsala, Sweden] was injected i.p. into the mice, and plasma glucose concentrations were measured before injection, and at 15, 30, 60, and 120 minutes after injection using blood glucose reagent strips (FreeStyle, Abbott, Alameda, CA).

Insulin Tolerance Tests

Insulin aspart (2 U/kg b.w.; NovoRapid; Novo Nordisk, Bagsvaerd, Denmark) was injected i.p., and plasma glucose levels were determined immediately before and 15, 30, 60, 90, 120, 180, 240, and 300 minutes after injection using blood glucose reagent strips.

Islet Endocrine Mass and Vascular Density

Pancreata from WT and *Mmp9*^{−/−} mice were retrieved, weighed, fixed in 10% (v/v) formaldehyde, and embedded in paraffin. Sections (5 μm thick) of the pancreata were stained with a guinea pig antibody for insulin (ICN Biomedicals, Aurora, OH),²³ and counterstained with hematoxylin. Some of the sections were also stained for endothelium with the lectin *Bandeiraea simplicifolia*-1 (BS-1; Sigma-Aldrich, St. Louis, MO).²³ From each animal, ≥5 tissue sections were systematically assessed to reflect all parts of the pancreas. The fraction of the pancreas that was composed of endocrine tissue was measured by a direct point-counting method²⁴ and used for calculation of the endocrine mass compensating for differences in pancreatic weight between animals. Islet vascular numbers were also counted in a microscope. The area of the investigated islets was determined using ImageJ software version 1.49g (NIH, Bethesda, MD).

Islet Isolation and Culture

Pancreatic islets from WT and *Mmp9*^{−/−} mice were isolated using a collagenase digestion protocol,²⁵ and maintained free floating in groups of 150 islets at 37°C (air/carbon dioxide, 95:5) overnight in 5 mL of tissue culture medium composed of RPMI1640 medium supplemented with 2 mmol/L L-glutamine, 11.1 mmol/L glucose, and 10% (v/v) fetal calf serum before the islet function assays (*cf.* below).

Glucose-Stimulated Insulin Release

After 3 to 4 days of culture, groups of 10 islets, from WT and from *Mmp9*^{−/−} mice, were transferred to vials containing Krebs-Ringer bicarbonate buffer supplemented with 10 mmol/L HEPES, 3 mg/mL bovine serum albumin (ICN Biomedicals, Santa Ana, CA), and 10 mmol/L glucosamine; hereafter referred to as KRBH buffer. The KRBH buffer contained 1.67 mmol/L D-glucose during the first hour of incubation at 37°C (oxygen/carbon dioxide, 95:5). The medium was then removed and replaced by KRBH supplemented with 16.7 mmol/L glucose and the islets were then incubated for a second hour. After this second incubation, the islets were harvested, following retrieval of the medium, and homogenized by sonication in 200 μL of redistilled water. A fraction of the homogenate was mixed with acid-ethanol [0.18 mol/L HCl in 95% (v/v) ethanol] from which insulin was extracted overnight. Thus, these measurements were performed after exposure to both low and high glucose in all islets. Insulin contents in incubation media and homogenates were determined by a mouse insulin enzyme-linked immunosorbent assay kit (Mercodia AB, Uppsala, Sweden).

(Pro)insulin Biosynthesis

Duplicate groups of 10 isolated islets from WT and *Mmp9*^{−/−} mice were size matched and incubated for 120 minutes in 100

μL of KRBH supplemented with 2 mg/mL bovine serum albumin, 16.7 mmol/L glucose, and L-[4,5-³H] leucine to measure the incorporation of tritiated leucine during (pro)-insulin and total protein biosynthesis. Assays were performed as described previously.¹⁶

Glucose Oxidation

Duplicate groups of 10 isolated islets from WT and *Mmp9*^{-/-} mice were size matched, incubated in 100 μL of KRBH supplemented with D-[U-¹⁴C] glucose and nonradioactive glucose to give a high (16.7 mmol/L) glucose concentration. The vials containing the islets were incubated for 90 minutes in a slow-shaking water bath at 37°C before the glucose oxidation was terminated with 100 μL of 0.05 mmol/L antimycin A (Sigma-Aldrich). Additionally 100 μL of 0.4 mol/L Na₂HPO₄ (pH 6.0) was injected to release the carbon dioxide formed. The carbon dioxide was trapped by the addition of Hyamine 10-X (New England Nuclear, Boston, MA) before another incubation period of 120 minutes at 37°C. Scintillation fluid (Ultima Gold, PerkinElmer, Waltham, MA) was added, and the radioactivity was measured in a liquid scintillation counter. The glucose oxidation rates were used as a measure of mitochondrial function.¹⁶

Diabetes Induction Model

Mice were injected i.p. with 40 mg/kg streptozotocin (Sigma-Aldrich) in citrate buffer on five consecutive days in a multiple low-dose streptozotocin (MLDS) protocol.²⁶ Blood glucose was monitored every third day using blood glucose reagent strips.

Islet Gene Expression

Total RNA was isolated from freshly isolated islets and with RNeasy Micro Plus kit (Qiagen, Hilden, Germany). The quality of the mRNA as measured by the absorbance ratio at 260 nm and 280 nm (NanoDrop; Thermo Fischer, Wilmington, DE) was above 1.85 for all samples. Conversion of RNA into complementary DNA was performed with the High Capacity cDNA kit (Life Technologies Europe, Stockholm, Sweden). Custom-ordered TaqMan Array Micro Fluidic Cards (exon-spanning probes were used when available) were run on an Applied Biosystems 7E900HT Fast Real Time PCR system (Foster City, CA). The gene expression was profiled through the comparative C_T method of relative quantification, with investigated genes (Supplemental Table S1) analyzed according to the manufacturer's instructions. β-Actin served as the reference gene.

Histology

Sections of formalin-fixed, paraffin-embedded mouse pancreata were stained using antibodies against VEGF-A (clone EP1176Y; Abcam, Cambridge, UK), collagen 4 (rabbit polyclonal, Abcam), laminin α5 (rabbit polyclonal, Abcam), and

laminin β2 (rabbit polyclonal; Novus Biologicals, Littleton, CO). Glycoproteins in the islets were stained by using the periodic acid-Schiff method. Staining intensity was assessed in 200× magnification micrographs using ImageJ software.

Transmission Electron Microscopy

For islet endothelial cell morphology and islet microvascular density, pancreata from WT and *Mmp9*^{-/-} mice were cut into small pieces and fixed in 2% glutaraldehyde and 1% paraformaldehyde. These were embedded in Agar 100 epoxy resin (Oxford Instruments, Stockholm, Sweden). Ultrathin sections (50 to 60 nm) were contrasted with uranyl acetate and lead citrate. Electron microscopy was performed using a FEI Tecnai G2 (Hillsboro, OR) transmission electron microscope at an accelerating voltage of 80 kV.

Islet Blood Flow

Mice were anesthetized by i.p. injections of avertin [2.5% (v/v) solution of 2,2,2-tribromoethanol (Sigma-Aldrich) in 2-methyl-2-butanol (Kemila, Stockholm, Sweden)] and placed on a heating pad to maintain body temperature. Animals were tracheostomized to facilitate respiration. Polyethylene catheters were inserted into the right carotid artery for blood pressure measurements and in the left femoral artery for blood sampling.

After a 15-minute resting period, blood flow measurements were performed using a microsphere technique as previously described.²⁷ Briefly, approximately 1.5×10^5 black nonradioactive microspheres (10-μm diameter; Triton microspheres, San Diego, CA) were injected through the carotid catheter during 5 seconds. For a total of 60 seconds, arterial blood was collected by free flow from the catheter in the femoral artery. The exact withdrawal rate was confirmed by weighing the sample. The pancreas, both adrenal glands, and samples (approximately 40 mg each) from the colon, duodenum, and kidney were removed and weighed. The number of microspheres in the samples, including pancreatic islets was counted as previously described after visualization of islet microspheres by a freeze-thawing technique.²⁸ The total number of islets and the total number of perfused islets were counted. Organ blood flow values were calculated according to the formula $Q_{org} = Q_{ref} \times N_{org}/N_{ref}$ where Q_{org} is the organ blood flow, Q_{ref} is withdrawal rate of the reference sample, N_{org} is the number of microspheres in the organ, and N_{ref} is the number of microspheres in the reference sample. The organ blood flow was expressed as milliliters per minute per gram tissue, whereas the islet perfusion was expressed as microliters per minute per gram pancreas. Adequate mixing of microspheres in the circulation was confirmed by comparing the blood flow values of the adrenal glands, which had to vary at most 10% within each animal.

Statistics

Values are expressed as mean ± SEM. Unpaired two-tailed *t*-tests or analysis of variance repeated measures were used

for comparison between groups. $P < 0.05$ was considered as statistically significant.

Results

Glucose Intolerance in *Mmp9*^{-/-} Mice

As previously described,²⁹ nonfasting blood glucose concentrations did not differ between *Mmp9*^{-/-} mice and WT mice (time 0 min) (Figure 1A). However, an i.p. glucose tolerance test revealed that the *Mmp9*^{-/-} mice had an impaired response to the glucose load (Figure 1A), which resulted in an increased area under the curve of plasma glucose values during the 120 minutes test (Figure 1B).

The Delayed Insulin Response in *Mmp9*^{-/-} Mice Is Islet-Related

To determine whether the delayed glucose handling in the *Mmp9*^{-/-} mice was related to islet defects or peripheral insulin resistance, peak insulin values during the i.p. glucose load were investigated and i.p. insulin tolerance tests were performed. When analyzing the serum insulin levels of the mice at the peak of insulin release (15 minutes) during the glucose tolerance test, the *Mmp9*^{-/-} mice had lower levels of

insulin (Figure 1C). By contrast, the response to the insulin challenge was similar in both genotypes (Figure 1D).

The Morphology of Islets in *Mmp9*^{-/-} Mice Is Unchanged

Mmp9^{-/-} have been shown to have a defect in their growth plates,³⁰ resulting in slight growth retardation and smaller body size (Supplemental Table S2³¹⁻⁴²). The size of the pancreas in relation to the total body weight was, however, unaffected by the gelatinase deficiency (WT versus *Mmp9*^{-/-}; $0.96 \pm 0.06\%$ versus $0.86 \pm 0.02\%$ pancreas weight of total b.w., $P = 0.14$). Matrix-degrading enzymes have also been postulated to affect migration of tissue cells during embryonal development, which may affect islet distribution and development. In the histological examination of pancreata from WT and *Mmp9*^{-/-} mice, we observed no difference in islet mass (Figure 1E), and a normal distribution of islets in the pancreas (Figure 1, F and G).

Impaired Glucose-Stimulated Insulin Release from *Mmp9*^{-/-} Mice Islet-Related

Because no reduction in islet mass could be observed, we investigated whether the defective insulin secretion of *Mmp9*^{-/-} mice *in vivo* remained *in vitro*, which would

Figure 1 Deficiency of matrix metalloproteinase-9 (*Mmp9*) results in a delayed response to glucose in mice. **A** and **B**: *Mmp9*^{-/-} mice have a delayed response to an i.p. glucose load (2.5 g/kg b.w.) compared to WT littermates, resulting in an increased area under the curve (**B**). **C**: The delay in the response to the glucose load is related to an impaired insulin release as measured by the serum insulin concentration 15 minutes after an i.p. glucose load. **D**: This difference in glucose uptake is not due to peripheral insulin intolerance because an i.p. insulin tolerance test (2 U/kg b.w.) shows no differences between the genotypes. **E**: The decreased insulin secretion in the *Mmp9*^{-/-} mice is not due to a decreased beta cell mass, as shown by histological examination of pancreatic tissue. **F** and **G**: Representative low-power magnification micrographs of insulin-stained pancreata from WT and *Mmp9*^{-/-} mice, respectively. $n = 8$ (**B** and **C**, mice per genotype); $n = 7$ (**D**, mice per genotype); $n = 5$ (**E**, mice per genotype); $n \geq 5$ tissue sections per pancreas (**E**, tissue sections per pancreas). * $P < 0.05$ (**A–C**). Scale bars: 100 μ m (**F** and **G**). Original magnification $\times 25$ (**F** and **G**). AUC, area under the curve.

indicate a change in beta-cell phenotype. Indeed, islets from *Mmp9*^{-/-} mice exhibited an impaired insulin release in response to a high glucose concentration when compared to islets of WT mice (Figure 2A). However, the content of insulin in the islets was similar in both the genotypes (Figure 2B), and so was glucose-stimulated (pro)insulin biosynthesis (Figure 2C), which indicate that the insulin-producing machinery was intact in *Mmp9*^{-/-} islets. Moreover, the mitochondrial function seemed normal, because the glucose oxidation rate did not differ between the genotypes (Figure 2D).

Mmp9^{-/-} Mice Are More Susceptible to MLDS Diabetes

MMP-9 has previously been suggested to be involved in development of diabetes.^{43,44} The multiple low-dose streptozotocin (MLDS) protocol is a way of mimicking an autoimmune destruction of the islets of Langerhans occurring during onset of type 1 diabetes.^{26,29} Mice of both genotypes were subjected to this protocol, and their blood glucose levels were monitored for 51 days. In our hands, this protocol gave mild diabetes with a slow onset. The blood glucose levels of the *Mmp9*^{-/-} mice were slightly, but consistently higher than the WT mice, and

differed significantly on 5 of 14 measuring points after the induction period (Figure 2E).

Abnormal Islet Gene and Protein Expression in *Mmp9*^{-/-} Mice

The islet-related differences between the two genotypes led us to investigate the expression of a number of genes related to glucose-sensing, glucose metabolism, insulin exocytosis, and vascular components of possible paracrine importance for the beta cells (Supplemental Table S1). The islets from the two genotypes did not differ in most of the assayed genes related to glucose metabolism and sensing. However, the mRNA levels of two proteins, lactate dehydrogenase A (LDH-A) and peroxisome proliferator-activated receptor gamma, coactivator 1 beta (PPARγC1β), were increased in the *Mmp9*^{-/-} (Figure 3A). Also, the mRNA for tryptophan hydroxylase 1 (Tph1) involved in serotonin synthesis, and islet amyloid polypeptide (Iapp) were increased in the *Mmp9*^{-/-} islets (Figure 3). A number of genes coupled to islet vascularity were assessed, and among these, the mRNA expression of two laminins (Lama5 and Lamb2), and plasminogen activator inhibitor 1 (Serpine1)

Figure 2 *Mmp9*^{-/-} islets of Langerhans exhibit impaired insulin release *in vitro* and an increased susceptibility to multiple low-dose streptozotocin (MLDS) diabetes *in vivo*. Isolated pancreatic islets were assessed *in vitro* to determine the origin of the impaired response to glucose. **A:** Islets from *Mmp9*^{-/-} show a decreased release of insulin in response to an increased glucose concentration (16.7 mmol/L versus 1.67 mmol/L) in the medium. **B–D:** However, insulin content of these islets is not different (**B**), as is neither the synthesis of (pro)insulin (**C**), nor the oxidation of glucose in the beta cells (**D**). **E:** In multiple low-dose streptozotocin treatments of mice, *Mmp9*^{-/-} mice are more susceptible to developing a more severe diabetes than their WT littermates. *n* = 5 (**A**, mice per genotype); *n* = 6 (**E**, mice per genotype). **P* < 0.05.

Figure 3 *Mmp9*^{-/-} pancreatic islets reveal compensatory transcriptional regulation at the gene and protein levels. The gene expression profile of whole *Mmp9*^{-/-} islets compared with WT islets, as determined by mRNA arrays ($n = 5$ mice per genotype). Genes related to the vasculature, the extracellular matrix, and beta cell metabolism are differently expressed. **A:** Genes that were detected with significantly different expression levels. [Supplemental Table S1](#) lists all genes assessed and $2^{-\Delta\Delta C_t}$ values. **B:** An IHC analysis of proteins related to the extracellular matrix was performed with the periodic acid-Schiff method. There are no differences in the amount of glycoproteins in the islets, or collagen IV, or VEGF-A present in the islets. The expressions of the matrix proteins laminin $\alpha 5$ and laminin $\beta 2$ are, however, significantly increased in *Mmp9*^{-/-} islets. **C:** Staining intensities are quantified. $n = 5$ (C, mice per genotype). * $P < 0.05$.

were augmented in *Mmp9*^{-/-} islets (Figure 3A). The expressions of laminin $\alpha 5$ and laminin $\beta 2$ were also shown to be increased on the protein level in immunohistochemical staining (Figure 3, B and C). Other matrix-related staining (periodic acid-Schiff and collagen IV) did not show any difference between the genotypes. Moreover, matrix metalloproteinase-7 (*Mmp7*) was upregulated in the *Mmp9*^{-/-} mice (Figure 3A). The vascular endothelial growth factor C (*Vegfc*) mRNA was also increased in *Mmp9* gene knockout mice (Figure 3A). All other genes that were assessed did not differ significantly between the genotypes.

Aberrant Vascular Morphology in *Mmp9*^{-/-} Islets

MMP-9 is known as a major contributor to angiogenesis.⁸ The intra-islet vascular densities of the *Mmp9*^{-/-} mice

were slightly decreased with regard to those found in WT mice (Figure 4A). When the ultrastructure of the capillary endothelial cells in the islets was studied by transmission electron microscopy, approximately 40% less fenestrae were observed in the *Mmp9*^{-/-} islets when compared to WT islets (Figure 4, B–F). These observations might suggest that the availability of VEGF-A is disturbed, ie, more VEGF-A is sequestered on the ECM. Anti-VEGF-A immunohistochemical staining, however, did not show any difference in staining intensity (Figure 3, B and C). The matrix degrading effect of MMP-9 could theoretically also influence and diminish the basement membrane between the beta cell and the endothelial cell, but no such effects were found when investigating islets by transmission electron microscopy. MMP-9 has also been implicated in the recruitment of pericytes to tumor microvasculature,⁴⁵ possibly affecting vascular stability and function. However, anti-NG-2 staining

Figure 4 *Mmp9*^{-/-} mice have aberrant pancreatic islet vasculature and an increased islet blood flow. Matrix metalloproteinase-9 (Mmp-9) is an angiogenic enzyme, but is redundant in the *Mmp9*^{-/-} mouse. **A:** However, in pancreatic islets, a slight decrease in intra-islet vascular density is seen in tissue sections. **B–F:** *Mmp9*^{-/-} mice have significantly fewer fenestrations in the islet capillaries (**B**) as seen in transmission electron microscopy images (**C–F**) of the islet vasculature. **Arrows** indicate fenestrae. **G:** The effect of *Mmp9* gene deletion on the islet blood flow was assessed by the microsphere technique. A threefold increase in blood flow in the *Mmp9*^{-/-} islets is observed. *n* = 5 (**A, C–F**, mice per genotype); *n* ≥ 5 (**A, C–F**, tissue sections per pancreas). **P* < 0.05. Scale bars: 0.5 μm (**C–F**). L, lumen; RBC, red blood cell.

of pancreatic sections revealed normal pericyte coverage in the islets of *Mmp9*^{-/-} mice (data not shown).

Blood Flow Is Markedly Increased in *Mmp9*^{-/-} Islets

Blood flow in pancreatic islets is normally tightly regulated and related to the sensing of glucose and the release of insulin by the beta cells. We investigated the impact of the detected vascular aberrations in the *Mmp9*^{-/-} islet capillaries for islet blood perfusion using the microsphere technique. Although the baseline blood glucose values, as well as mean arterial blood pressure (data not shown) were similar in the WT and *Mmp9*^{-/-} mice, the islet blood flow in the *Mmp9*^{-/-} mice was increased approximately threefold at baseline conditions (Figure 4G).

Discussion

The matrix-degrading enzyme gelatinase B/MMP-9 has a multitude of functions in development and homeostasis, as well as in different pathologies.^{6,7} Here, we investigated the enigmatic role of this enzyme on the function of pancreatic islets. *Mmp9*^{-/-} mice were shown to have an impaired insulin response to a glucose load. This impairment was found to depend on a change in beta cell phenotype, because *Mmp9*^{-/-} islets exhibited a defective insulin secretion in response to a glucose challenge both *in vivo* and *in vitro*. With the use of

gene array studies we were able to demonstrate significant changes in a number of pancreatic marker molecules, which were induced by the gene deletion. Moreover, substantial structural and functional changes in the islet vasculature were observed in *Mmp9*^{-/-} mice.

The role of the islet matrix for beta cell function has not yet been extensively studied. However, components of the islet basement membrane such as laminins,^{15,16} as well as components of, or molecules sequestered on the islet matrix, eg, heparan sulfate⁴⁶ and thrombospondin-1¹⁷ have been reported to be of high importance for support of beta cell differentiation, function, and proliferation. Moreover, VEGF-A, which is secreted by the beta cells and important for islet vascular maintenance and phenotype, is to a large proportion bound to the islet matrix. The depletion of MMP-9 most certainly decreases the islet matrix turnover, and thereby reduces the availability of immobilized molecules, such as growth factors. Indeed, the loss of MMP-9 function was accompanied by a decreased islet vascularization, and a marked reduction in the numbers of fenestrae in the capillaries. This finding indicates a lowered availability of the beta-cell-secreted VEGF-A for the islet endothelium in *Mmp9*^{-/-} mice. Because the VEGF-A expression per se was not altered, this effect might be related to the previously described action of MMP-9 to release VEGF-A from the extracellular matrix. We were not, however, able to see any difference in the amount of VEGF-A sequestered in the islets using an immunohistochemical method. Because the islets are expressing

VEGF-A at high levels already at the normal state, a difference might be difficult to be demonstrated with this approach. Nevertheless, in contrast to the studies with selectively reduced VEGF availability by interference at the transcriptional level,^{20,47} we observed concomitant changes in beta cell phenotype. This suggests that a normal islet matrix turnover is important, not only to maintain islet vascularity, but also for beta cell function in response to a glucose challenge.

A faulty insulin release machinery would cause delayed insulin secretion in response to glucose. However, we could not see any alterations at the transcriptional level of proteins previously described to be involved in insulin exocytosis,⁴⁸ eg, Syntaxin 1A, Syntaxin 4A, and Snap25 in the *Mmp9*^{-/-} islets. In addition, functional tests did not reveal any defects in the (pro)insulin biosynthesis nor in the mitochondria (the latter assessed by glucose oxidation rates). However, up-regulation of *Pparg1b* and *Ldha* in *Mmp9*^{-/-} islets indicate induction of non-oxidative glucose metabolism.⁴⁹ Up-regulation of lactate dehydrogenase A has previously been shown to perturb the normal glucose sensing of the beta cell, resulting in a deteriorated glucose-stimulated insulin secretion response.^{50,51}

Tryptophan hydroxylase-1 (TPH-1) is important both for insulin secretion by serotonylation of GTPases⁵² and for beta cell mass expansion during pregnancy.⁵³ The observed up-regulated expression of *Tph1* in *Mmp9*^{-/-} mice would, therefore, seem paradoxical, but may reflect positive effects of a lowered islet matrix turnover or be a compensatory phenomenon. Also, *Iapp* was up-regulated in *Mmp9*^{-/-} mice. MMP-9 has been implicated in the degradation of human IAPP (islet amyloid polypeptide⁴³), which may suggest that the *Iapp* up-regulation was a compensatory phenomenon. However, IAPP per se seems to have mild or no physiological importance for islet function.^{54,55}

In addition, MMP-9 in the pancreatic islet may cleave and release components in the vascular basement membrane of importance for the beta cells. We therefore first hypothesized that the basement membrane would be thickened by the lack of MMP-9, but ultrastructural examination of the islet capillaries revealed that this was not the case. Noteworthy, the gene expression of *Mmp7* was up-regulated, and this proteinase and others might have compensated, at least partly, for the lack of MMP-9. Nevertheless, small differences in protease activity may influence the turnover and accumulation of MMP-9-substrates such as collagen IV and laminins. In fact, we observed an increased expression of two laminin genes (*Lama5* and *Lamb2*), congruent with an unbalanced turnover in the basement membrane.

Of note, several of the genes that were found to be up-regulated in the *Mmp9*^{-/-} islets are well-known substrates to MMP-9. Serpins,^{56,57} laminins,^{58,59} and IAPP⁴³ are cleaved by this enzyme, and cleavage products from LDH-A have also been found in cell supernatants following MMP-9 activity.⁶⁰ This suggests a direct link between the gene deletion and the compensatory changes in gene expression.

Islet blood flow is normally high and tightly regulated to meet the metabolic demands for insulin secretion at any time.^{12,61} Despite a lower vascular density, we recorded a threefold higher islet blood flow in *Mmp9*^{-/-} islets when compared to WT islets at baseline conditions, ie, at similar plasma glucose values. This may reflect a compensatory phenomenon for the lower vascular density with a blood flow increase mediated by ATP metabolites at a condition with a less optimal oxygen transport to tissue.⁶²

The up-regulation of MMP-9 in the pancreas has been demonstrated to be a diabetogenic factor in human pancreatitis⁶³ and in experimental models of diabetes.²⁹ Moreover, increased circulating concentrations of MMP-9 can be measured in type 1 diabetic patients.⁶⁴ However, *Mmp9*^{-/-} mice in the present study were observed to have a paradoxically increased susceptibility to MLDS diabetes. This might be due to the impaired beta cell function of *Mmp9*^{-/-} mice observed already during basal conditions. Moreover, the threefold higher blood perfusion of *Mmp9*^{-/-} islets would be expected to increase the delivery of streptozotocin to these islets thereby increasing their vulnerability.

Previous studies of the influence of MMP-9 on pancreas physiology did not address the development and function of the normal mouse pancreas or any compensation mechanism by the gene deletion. In addition, the normal development of mouse pancreas has been studied⁵ and, also in that study, compensation mechanisms were not yet discovered.⁵ In the current study, the observed alterations were made in a nonleaky *Mmp9* knockout mouse and are reflecting convincingly true compensatory mechanisms (Supplemental Table S2). The changes seen in this study are not based on tissue reorganization by an acute insult, but rather are the result of homeostatic long-term events. Therefore, our data are also relevant for those future studies in which long-term MMP-9 inhibition will be used. It will be critical to monitor glycemia, diabetes induction, and eventually, islet blood flow changes in such studies.

In conclusion, this study presents an in-depth investigation of the effects of the loss of MMP-9 function on the islets of Langerhans and complements previous work.⁵ A deteriorated beta cell function was observed, primarily as a result of a shift in the beta cell phenotype, but also because of islet vascular aberrations. These effects are likely to reflect the importance of a normal islet matrix turnover exerted by MMP-9, and concomitant release and proteolysis of paracrine signaling proteins sequestered on the matrix.

Acknowledgments

We thank Birgitta Bodin, Ing-Britt Hallgren, Astrid Nordin, Malin Olsen, Zhanchun Li (Uppsala University, Sweden), and Anders Ahlander (SciLifeLab, Uppsala University) for skilled technical assistance; Greet Thijs (Rega Institute for Medical Research, University of Leuven, Belgium) for

excellent care for the backcrosses and maintenance of the *Mmp9*^{-/-} mouse colonies.

G.C. designed experiments, performed research, analyzed data, and wrote the manuscript; T.W. performed research and analyzed data; M.S. performed research; G.O. contributed mice and to the discussion of the results and writing the manuscript; P.-O.C. initiated the study, designed experiments, and wrote the manuscript; M.P. designed experiments, analyzed data, wrote the manuscript, and supervised the research project. All authors revised the manuscript critically for intellectual content and approved the final version of the paper.

Supplemental Data

Supplemental material for this article can be found at <http://dx.doi.org/10.1016/j.ajpath.2014.12.009>.

References

- Tomita T, Iwata K: Gelatinases and inhibitors of gelatinases in pancreatic islets and islet cell tumors. *Mod Pathol* 1997, 10:47–54
- Barro C, Zaoui P, Morel F, Benhamou PY: Matrix metalloproteinase expression in rat pancreatic islets. *Pancreas* 1998, 17:378–382
- Miralles F, Battelino T, Czernichow P, Scharfmann R: TGF- β plays a key role in morphogenesis of the pancreatic islets of Langerhans by controlling the activity of the matrix metalloproteinase MMP-2. *J Cell Biol* 1998, 143:827–836
- Miettinen PJ, Huotari M, Koivisto T, Ustinov J, Palgi J, Rasilainen S, Lehtonen E, Keski-Oja J, Otonkoski T: Impaired migration and delayed differentiation of pancreatic islet cells in mice lacking EGF-receptors. *Development* 2000, 127:2617–2627
- Perez SE, Cano DA, Dao-Pick T, Rougier JP, Werb Z, Hebrok M: Matrix metalloproteinases 2 and 9 are dispensable for pancreatic islet formation and function in vivo. *Diabetes* 2005, 54:694–701
- Van den Steen PE, Dubois B, Nelissen I, Rudd PM, Dwek RA, Opdenakker G: Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9). *Crit Rev Biochem Mol Biol* 2002, 37:375–536
- Vandooren J, Van den Steen PE, Opdenakker G: Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9): the next decade. *Crit Rev Biochem Mol Biol* 2013, 48:222–272
- Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, Tanzawa K, Thorpe P, Itohara S, Werb Z, Hanahan D: Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. *Nat Cell Biol* 2000, 2:737–744
- Olsson R, Maxhuni A, Carlsson PO: Revascularization of transplanted pancreatic islets following culture with stimulators of angiogenesis. *Transplantation* 2006, 82:340–347
- Christoffersson G, Vagesjo E, Vandooren J, Liden M, Massena S, Reinert RB, Brissova M, Powers AC, Opdenakker G, Phillipson M: VEGF-A recruits a proangiogenic MMP-9-delivering neutrophil subset that induces angiogenesis in transplanted hypoxic tissue. *Blood* 2012, 120:4653–4662
- Lifson N, Lassa CV, Dixit PK: Relation between blood flow and morphology in islet organ of rat pancreas. *Am J Physiol* 1985, 249:E43–E48
- Jansson L: The regulation of pancreatic islet blood flow. *Diabetes Metab Rev* 1994, 10:407–416
- Bonner-Weir S, Orci L: New perspectives on the microvasculature of the islets of Langerhans in the rat. *Diabetes* 1982, 31:883–889
- Peiris H, Bonder CS, Coates PT, Keating DJ, Jessup CF: The beta-cell/EC axis: how do islet cells talk to each other? *Diabetes* 2014, 63:3–11
- Nikolova G, Jabs N, Konstantinova I, Domogatskaya A, Tryggvason K, Sorokin L, Fassler R, Gu G, Gerber HP, Ferrara N, Melton DA, Lammert E: The vascular basement membrane: a niche for insulin gene expression and Beta cell proliferation. *Dev Cell* 2006, 10:397–405
- Johansson A, Lau J, Sandberg M, Borg LA, Magnusson PU, Carlsson PO: Endothelial cell signalling supports pancreatic beta cell function in the rat. *Diabetologia* 2009, 52:2385–2394
- Drott CJ, Olerud J, Emanuelsson H, Christoffersson G, Carlsson PO: Sustained Beta-cell dysfunction but normalized islet mass in aged thrombospondin-1 deficient mice. *PLoS One* 2012, 7:e47451
- Johansson M, Mattsson G, Andersson A, Jansson L, Carlsson PO: Islet endothelial cells and pancreatic beta-cell proliferation: studies in vitro and during pregnancy in adult rats. *Endocrinology* 2006, 147:2315–2324
- Lammert E, Gu G, McLaughlin M, Brown D, Brekken R, Murtaugh LC, Gerber HP, Ferrara N, Melton DA: Role of VEGF-A in vascularization of pancreatic islets. *Curr Biol* 2003, 13:1070–1074
- Reinert RB, Brissova M, Shostak A, Pan FC, Poffenberger G, Cai Q, Hundemer GL, Kantz J, Thompson CS, Dai C, McGuinness OP, Powers AC: Vascular endothelial growth factor- α and islet vascularization are necessary in developing, but not adult, pancreatic islets. *Diabetes* 2013, 62:4154–4164
- Kamba T, Tam BY, Hashizume H, Haskell A, Sennino B, Mancuso MR, Norberg SM, O'Brien SM, Davis RB, Gowen LC, Anderson KD, Thurston G, Joho S, Springer ML, Kuo CJ, McDonald DM: VEGF-dependent plasticity of fenestrated capillaries in the normal adult microvasculature. *Am J Physiol Heart Circ Physiol* 2006, 290:H560–H576
- Dubois B, Masure S, Hurtenbach U, Paemen L, Heremans H, van den Oord J, Sciort R, Meinhardt T, Hämmerling G, Opdenakker G, Arnold B: Resistance of young gelatinase B-deficient mice to experimental autoimmune encephalomyelitis and necrotizing tail lesions. *J Clin Invest* 1999, 104:1507–1515
- Jansson G, Jansson L, Carlsson PO: Decreased vascular density in mouse pancreatic islets after transplantation. *Diabetes* 2002, 51:1362–1366
- Weibel ER: Morphometry of the human lung: the state of the art after two decades. *Bull Eur Physiopathol Respir* 1979, 15:999–1013
- Andersson A: Isolated mouse pancreatic islets in culture: effects of serum and different culture media on the insulin production of the islets. *Diabetologia* 1978, 14:397–404
- Carlsson PO, Flodstrom M, Sandler S: Islet blood flow in multiple low dose streptozotocin-treated wild-type and inducible nitric oxide synthase-deficient mice. *Endocrinology* 2000, 141:2752–2757
- Kampf C, Bodin B, Kallskog O, Carlsson C, Jansson L: Marked increase in white adipose tissue blood perfusion in the type 2 diabetic GK rat. *Diabetes* 2005, 54:2620–2627
- Jansson L, Hellerstrom C: A rapid method of visualizing the pancreatic islets for studies of islet capillary blood flow using non-radioactive microspheres. *Acta Physiol Scand* 1981, 113:371–374
- Descamps FJ, Martens E, Ballaux F, Geboes K, Opdenakker G: In vivo activation of gelatinase B/MMP-9 by trypsin in acute pancreatitis is a permissive factor in streptozotocin-induced diabetes. *J Pathol* 2004, 204:555–561
- Vu TH, Shipley JM, Bergers G, Berger JE, Helms JA, Hanahan D, Shapiro SD, Senior RM, Werb Z: MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. *Cell* 1998, 93:411–422
- Cauwe B, Martens E, Sagaert X, Dillen C, Geurts N, Li S, Mertens J, Thijs G, Van den Steen PE, Heremans H, De Vos R, Blockmans D,

- Arnold B, Opdenakker G: Deficiency of gelatinase B/MMP-9 aggravates lpr-induced lymphoproliferation and lupus-like systemic autoimmune disease. *J Autoimmun* 2011, 36:239–252
32. Dubois B, Arnold B, Opdenakker G: Gelatinase B deficiency impairs reproduction. *J Clin Invest* 2000, 106:627–628
 33. Plaks V, Rinkenberger J, Dai J, Flannery M, Sund M, Kanasaki K, Ni W, Kalluri R, Werb Z: Matrix metalloproteinase-9 deficiency phenocopies features of preeclampsia and intrauterine growth restriction. *Proc Natl Acad Sci U S A* 2013, 110:11109–11114
 34. Camp TM, Tyagi SC, Senior RM, Hayden MR, Tyagi SC: Gelatinase B(MMP-9) an apoptotic factor in diabetic transgenic mice. *Diabetologia* 2003, 46:1438–1445
 35. Descamps FJ, Martens E, Proost P, Starckx S, Van den Steen PE, Van Damme J, Opdenakker G: Gelatinase B/matrix metalloproteinase-9 provokes cataract by cleaving lens betaB1 crystallin. *FASEB J* 2005, 19:29–35
 36. Kowluru RA, Mohammad G, dos Santos JM, Zhong Q: Abrogation of MMP-9 gene protects against the development of retinopathy in diabetic mice by preventing mitochondrial damage. *Diabetes* 2011, 60:3023–3033
 37. Mishra PK, Chavali V, Metreveli N, Tyagi SC: Ablation of MMP9 induces survival and differentiation of cardiac stem cells into cardiomyocytes in the heart of diabetics: a role of extracellular matrix. *Can J Physiol Pharmacol* 2012, 90:353–360
 38. Lingwal N, Padmasekar M, Samikannu B, Bretzel RG, Preissner KT, Linn T: Inhibition of gelatinase B (matrix metalloproteinase-9) activity reduces cellular inflammation and restores function of transplanted pancreatic islets. *Diabetes* 2012, 61:2045–2053
 39. Kundu S, Pushpakumar SB, Tyagi A, Coley D, Sen U: Hydrogen sulfide deficiency and diabetic renal remodeling: role of matrix metalloproteinase-9. *Am J Physiol Endocrinol Metab* 2013, 304: E1365–E1378
 40. Li SY, Huang PH, Yang AH, Tarn DC, Yang WC, Lin CC, Chen JW, Schmid-Schonbein G, Lin SJ: Matrix metalloproteinase-9 deficiency attenuates diabetic nephropathy by modulation of podocyte functions and dedifferentiation. *Kidney Int* 2014, 86:358–369
 41. Mohammad G, Vandooren J, Siddiquei MM, Martens E, Abu El-Asrar AM, Opdenakker G: Functional links between gelatinase B/matrix metalloproteinase-9 and prominin-1/CD133 in diabetic retinal vasculopathy and neuropathy. *Prog Retin Eye Res* 2014, 43:76–91
 42. Hu J, Van den Steen PE, Sang QX, Opdenakker G: Matrix metalloproteinase inhibitors as therapy for inflammatory and vascular diseases. *Nat Rev Drug Discov* 2007, 6:480–498
 43. Aston-Mourney K, Zraika S, Udayasankar J, Subramanian SL, Green PS, Kahn SE, Hull RL: Matrix metalloproteinase-9 reduces islet amyloid formation by degrading islet amyloid polypeptide. *J Biol Chem* 2013, 288:3553–3559
 44. Jackson AM, Kanak MA, Grishman EK, Chaussabel D, Levy MF, Naziruddin B: Gene expression changes in human islets exposed to type 1 diabetic serum. *Islets* 2012, 4:312–319
 45. Du R, Lu KV, Petritsch C, Liu P, Ganss R, Passegue E, Song H, Vandenberg S, Johnson RS, Werb Z, Bergers G: HIF1alpha induces the recruitment of bone marrow-derived vascular modulatory cells to regulate tumor angiogenesis and invasion. *Cancer Cell* 2008, 13: 206–220
 46. Ziolkowski AF, Popp SK, Freeman C, Parish CR, Simeonovic CJ: Heparan sulfate and heparanase play key roles in mouse beta cell survival and autoimmune diabetes. *J Clin Invest* 2012, 122: 132–141
 47. D'Hoker J, De Leu N, Heremans Y, Baeyens L, Minami K, Ying C, Lavens A, Chintinne M, Stange G, Magenheimer J, Swisa A, Martens G, Pipeleers D, van de Castele M, Seino S, Keshet E, Dor Y, Heimberg H: Conditional hypovascularization and hypoxia in islets do not overtly influence adult beta-cell mass or function. *Diabetes* 2013, 62:4165–4173
 48. Jewell JL, Oh E, Thurmond DC: Exocytosis mechanisms underlying insulin release and glucose uptake: conserved roles for Munc18c and syntaxin 4. *Am J Physiol Regul Integr Comp Physiol* 2010, 298: R517–R531
 49. Handschin C, Spiegelman BM: Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. *Endocr Rev* 2006, 27:728–735
 50. Ishihara H, Wang H, Drewes LR, Wollheim CB: Overexpression of monocarboxylate transporter and lactate dehydrogenase alters insulin secretory responses to pyruvate and lactate in beta cells. *J Clin Invest* 1999, 104:1621–1629
 51. Sekine N, Cirulli V, Regazzi R, Brown LJ, Gine E, Tamarit-Rodriguez J, Girotti M, Marie S, MacDonald MJ, Wollheim CB, Rutter GA: Low lactate dehydrogenase and high mitochondrial glycerol phosphate dehydrogenase in pancreatic beta-cells. Potential role in nutrient sensing. *J Biol Chem* 1994, 269: 4895–4902
 52. Paulmann N, Grohmann M, Voigt JP, Bert B, Vowinkel J, Bader M, Skelin M, Jevsek M, Fink H, Rupnik M, Walther DJ: Intracellular serotonin modulates insulin secretion from pancreatic beta-cells by protein serotonylation. *PLoS Biol* 2009, 7: e1000229
 53. Kim H, Toyofuku Y, Lynn FC, Chak E, Uchida T, Mizukami H, Fujitani Y, Kawamori R, Miyatsuka T, Kosaka Y, Yang K, Honig G, van der Hart M, Kishimoto N, Wang J, Yagihashi S, Tecott LH, Watada H, German MS: Serotonin regulates pancreatic beta cell mass during pregnancy. *Nat Med* 2010, 16:804–808
 54. Gebre-Medhin S, Mulder H, Pekny M, Westermark G, Tornell J, Westermark P, Sundler F, Ahren B, Betsholtz C: Increased insulin secretion and glucose tolerance in mice lacking islet amyloid polypeptide (amylin). *Biochem Biophys Res Commun* 1998, 250: 271–277
 55. Carlsson PO, Karlsson E, Mulder H, Gebre-Medhin S: Unaltered pancreatic islet blood perfusion in islet amyloid polypeptide-deficient mice. *Eur J Endocrinol* 2002, 146:107–112
 56. Liu X, Zhou X, Shapiro SD, Shipley JM, Twining SS, Diaz LA, Senior RM, Werb Z: The serpin alpha1-proteinase inhibitor is a critical substrate for gelatinase B/MMP-9 in vivo. *Cell* 2000, 102: 647–655
 57. McKee CM, Xu D, Kessler BM, Muschel RJ: Proteomic analysis reveals a proteolytic feedback loop in murine seminal fluid. *Prostate* 2013, 73:1427–1440
 58. Gu Z, Cui J, Brown S, Fridman R, Mobashery S, Strongin AY, Lipton SA: A highly specific inhibitor of matrix metalloproteinase-9 rescues laminin from proteolysis and neurons from apoptosis in transient focal cerebral ischemia. *J Neurosci* 2005, 25:6401–6408
 59. Zamilpa R, Lopez EF, Chiao YA, Dai Q, Escobar GP, Hakala K, Weintraub ST, Lindsey ML: Proteomic analysis identifies in vivo candidate matrix metalloproteinase-9 substrates in the left ventricle post-myocardial infarction. *Proteomics* 2010, 10:2214–2223
 60. Prudova A, auf dem Keller U, Butler GS, Overall CM: Multiplex N-terminome analysis of MMP-2 and MMP-9 substrate degradomes by iTRAQ-TAILS quantitative proteomics. *Mol Cell Proteomics* 2010, 9:984–911
 61. Jansson L, Hellerstrom C: Stimulation by glucose of the blood flow to the pancreatic islets of the rat. *Diabetologia* 1983, 25: 45–50
 62. Carlsson PO, Olsson R, Kallskog O, Bodin B, Andersson A, Jansson L: Glucose-induced islet blood flow increase in rats: interaction between nervous and metabolic mediators. *Am J Physiol Endocrinol Metab* 2002, 283:E457–E464
 63. Descamps FJ, Van den Steen PE, Martens E, Ballaux F, Geboes K, Opdenakker G: Gelatinase B is diabetogenic in acute and chronic pancreatitis by cleaving insulin. *FASEB J* 2003, 17:887–889
 64. Jacqueminet S, Ben Abdesselam O, Chapman MJ, Nicolay N, Foglietti MJ, Grimaldi A, Beaudeau JL: Elevated circulating levels of matrix metalloproteinase-9 in type 1 diabetic patients with and without retinopathy. *Clin Chim Acta* 2006, 367: 103–107