
Suppl. materials and methods:

Reagents

The following antibodies were used in the current study: rabbit polyclonal antibody to Wilms' tumor-1, rabbit polyclonal antibody to synaptopodin and GLEPP1 (Santa Cruz, Heidelberg, Germany); rabbit polyclonal antibodies to β-actin, TGF-β, and phosphorylated-Smad (Cell Signaling, Frankfurt, Germany); rabbit anti-complement 5b-9 (MAC) polyclonal antibody (Merck, Nottingham, UK), unlabelled or FITC labelled goat polyclonal antibody to complement factor C3 (Cappel, Ohio, OH, USA); rabbit polyclonal antibody to HRP or FITC conjugated secondary antibodies to mouse and rabbit IgG (Abcam, Cambridge, UK).

The following reagents were obtained from Sigma-Aldrich, Taufkirchen, Germany: Streptozocin, 1% collagen, insulin-transferin-selenium (ITS) liquid media supplement, DMEM, HOECHST 33258 and Trizol.

Other reagents used in the current study: FCS (PAA, Germany), RevertAid™ H Minus First Strand cDNA Synthesis kit (Fermentas, Heidelberg, Germany), Enzygnost® TAT micro Elisa kit (Siemens Healthcare Diagnostics, Germany); mouse albumin elisa quantitation kit (Bethyl Laboratories, TX, USA); DMEM, trypsin-EDTA, fetal bovine serum (FBS), and HEPES (PAA laboratories, Pasching, Austria); Interferon γ (cell sciences, Canton, MA); protease inhibitor cocktail (Roche diagnostics GmbH, Mannheim, Germany); Phusion® High-Fidelity Tag Polymerase PCR kit (Finnzymes, Espoo, Finland); pcDNA™3.1/V5-His TOPO and Lipofectamine™ 2000 (Invitrogen, Karlsruhe, Germany); Vectashield mounting medium, DAB
(Vector Laboratories, Burlington, CA, USA); PVDF membrane (Millipore GmbH, Schwalbach, Germany); enhanced chemiluminescence (ECL) reagent (Amersham, Piscataway, NJ, USA).

**Immunoblotting**

Kidney tissue samples of renal cortex were prepared by removing the outer 1/3 of the kidney using a scalpel and a dissecting microscope and immunoblotting was performed as described (1). To compare and quantify levels of proteins, the density of each band was measured using Image J software. Equal loading was confirmed by actin immunoblot.

**RT-PCR**

RNA was isolated using Trizol reagent according to manufacturer’s instructions. cDNA was generated using 1 µg total RNA following treatment with DNase (1U/1µg RNA) followed by reverse transcription using RevertAid™ Reverse Transcriptase. PCR primers used for expression TM analyses are as follows: forward 5’-TGC TTC TGC TAT GAT GGC TAT GAG TT-3’, reverse 5’-GGG GTC ACA GTC TTT GCT AAT C TG A-3’. The PCR products were separated on a 1.8% agarose gel and visualized by ethidium bromide staining. Expression was normalized to β-actin. Reactions lacking reverse transcriptase served as negative controls.

**Analyses of TAT complexes, anti-fXa activity, and HMGB1**

We quantified TAT (thrombin-antithrombin) complexes and HMGB1 plasma levels using commercially available ELISAs as previously described (2, 3). The tail bleeding time was determined as previously described (2). Briefly, immobilized tails were cut at a given diameter. Blood drops were collected on Whatman paper (No 1001-185) at least every 30 sec. for a maximum of 20 min. The time until cessation of bleeding was determined. Determination of anti-fXa activity in mouse plasma samples was performed on an automated platform (STA-R Evolution® Expert Series Hemostasis System, Stago, Düsseldorf, Germany) using Coamatic®
reagents (Haemochrom Diagnostica, Essen, Germany) and calibrators for either fondaparinux
or enoxaparin (Technoclone, Vienna, Austria).

References:
ameliorates glucose-induced podocyte apoptosis: protective effect of factor V Leiden in
2. Isermann B, Vinnikov IA, Madhusudhan T, et al. Activated protein C protects against
3. Andrassy M, Volz HC, Igwe JC, et al. High-mobility group box-1 in ischemia-
**Supplementary Figure 1**

(a) Representative images of PAS stained kidneys at low magnification, showing multiple glomeruli and tubuli.

(b,c): Representative images of Masson's trichrome stainings (b) and bar graph summarizing results of Masson's trichrome-stained area (c; ≥50 glomeruli of at least 7 different mice). Scale bar: 40 μm (a,b); C: non-diabetic control mice, open bars; DM: diabetic mice, black bars; mean value ± SEM; *: P<0.05, **: P<0.01.

**Loss of TM’s lectin-like domain aggravates experimental diabetic nephropathy**

- **a**: representative images of PAS stained kidneys at low magnification, showing multiple glomeruli and tubuli.
- **b**, **c**: representative images of Masson's trichrome stainings (b) and bar graph summarizing results of Masson's trichrome-stained area (c; ≥50 glomeruli of at least 7 different mice). Scale bar: 40 μm (a,b); C: non-diabetic control mice, open bars; DM: diabetic mice, black bars; mean value ± SEM; *: P<0.05, **: P<0.01.
**Supplementary Figure 2**

**a**, **b:** Cleaved caspase-3, immunohistochemically detected, is increased to a similar extent in diabetic TM^{WT/WT} and diabetic TM^{LeD/LeD} mice. Representative images (a, cleaved caspase-3 detected by HRP-DAB reaction, brown; hematoxylin counterstain, blue). The frequency of cleaved caspase-3-positive cells per 50 glomerular profiles was determined (b, n=10 mice for each group).

**c**, **d:** Expression of apoptosis regulators in renal cortex extracts. Bar graph (top) and representative immunoblot (bottom) showing p53 (c) and Bax (d) expression in renal cortex tissue samples (n=7 for each group).

Scale bar: 20 µm (a); C: non-diabetic control mice, open bars; DM: diabetic mice, black bars; mean value ± SEM; ∗: P<0.05, ∗∗: P<0.01.

**TM's lectin-like domain regulates diabetic nephropathy independent of apoptosis**

The lectin-like domain of TM regulates diabetic nephropathy independently of apoptosis. This is evidenced by the increased expression of cleaved caspase-3 in diabetic mice compared to control, as shown in the immunohistochemical images (a). The frequency of cleaved caspase-3-positive cells per 50 glomerular profiles is higher in diabetic mice, as depicted in bar graph (b). The relative expression levels of p53 and Bax, as determined by immunoblot analysis, further support this finding (c and d). These results indicate that the lectin-like domain of TM plays a pivotal role in the development of diabetic nephropathy, distinct from the effects of apoptosis.
Supplementary Figure 3

a,b: Using immunohistochemical staining no difference of tissue HMGB1 is detected in TM^{WT} or TM^{Le/Ld} mice without or with diabetes. Representative images of immunohistochemical stained section (a, HMGB1 detected by HRP-DAB reaction, brown; hematoxylin counterstain, blue) and bar graph summarizing results using computerized image analyses (b, n>=10 for each group).

c,d: Tissue (determined by immunoblotting) and plasma levels (determined by ELISA) of HMGB1 are comparable in TM^{WT} or TM^{Le/Ld} mice without or with diabetes. Bar graph summarizing results (c, d, n=7 for each group).

Scale bar: 20 μm (a); C: non-diabetic control mice, open bars; DM: diabetic mice, black bars; mean value ± SEM.
Effect of enoxaparin and fondaparinux on complement activation.

Enoxaparin (open bars), but not fondaparinux (black bars) reduces complement activation in human (A) and background matched mouse (B) plasma samples, as determined by the red cell hemolysis assay.

The red cell hemolysis assay was conducted employing an established protocol used in the daily routine by one of the co-authors (Mi.Ki., reference 1 below). Briefly, freshly collected and carefully washed (3 times) rabbit red blood cells (RBC) were incubated with goat anti-rabbit RBC antiserum (20 min, 37 °C, Cappel, Ohio, OH, USA). RBCs were then again washed (3 times) and resuspended at a concentration of 1.5 x 10⁸ cells/ml. Human (a) or mouse (b) serum (300µl) with different dilution of anticoagulants were then added to 50 µl of antibody incubated RBCs in the presence of 0.15 mM Ca²⁺ and 1 mM Mg²⁺. For a positive control 300 µl water and for a negative control 300 µl assay buffer were added. Cells were incubated at 37°C for 60 min, then centrifuged (1800 RPM for 10 minutes), and the absorbance (415 nm) in the supernatants was determined within 15 minutes after centrifugation. The hemolysis rate was calculated by comparing the sample OD with the positive control (100%) after subtracting the value of the negative control.

Reference:
Supplementary Figure 5

a: representative images of PAS stained kidneys at low magnification, showing multiple glomeruli and tubuli.
b,c: representative images of Masson’s trichrome stainings (b) and bar graph summarizing results of Masson's trichrome-stained area (c; ≥50 glomeruli of at least 7 different mice). Scale bar: 40 μm (a,b); DM: diabetic TMLeD/LeD mice, black bars; DM + Enox: diabetic TMLeD/LeD mice treated with enoxaparin, grey bars; DM + Fonda: diabetic TMLeD/LeD mice treated with fondaparinux, speckled bars; mean value ± SEM; *: P<0.05, **: P<0.01.

Enoxaparin, but not fondaparinux, ameliorates nephropathy in diabetic TMLeD/LeD mice