TIMP-1 DEFICIENCY ASSOCIATED WITH REDUCED RENAL EGR-1 EXPRESSION AMELIORATES DIABETIC NEPHROPATHY IN MICE

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BACKGROUND

Diabetic nephropathy is associated with functional imbalance of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs). Renal TIMP-1 overexpression has been reported in both experimental and human diabetic nephropathy [1,2].

EGR1 is an early response transcription factor in fibrosis regulating collagen synthesis [3,4]. However, its association with diabetes or TIMP-1 has not been investigated yet.

We aimed to investigate the progression of nephropathy in type-1 diabetic TIMP-1 knockout (KO) mice and its association with the profibrotic transcription factor EGR1.

METHODS

Animal model of type-1 diabetes:

Diabetes was induced in 6 week-old male C57bl6-Timp1 knockout (Timp1) and C57Bl6 wild type (WT) mice weighing 20-22 grams with daily intraperitoneal streptozotocin (50 mg/kg/day) injections for 5 consecutive days.

Experimental groups:

1) diabetic Timp1 KO (Timp1, n=8)
2) diabetic wild type control (WT, n=5)

Fasting blood glucose was monitored once weekly.

After 8 weeks, kidneys were analyzed for histology and mRNA expression levels as follows.

Performed analyses:

- Urinary protein/creatinin ratio
- serum urea levels
- Glomerulosclerosis index
- Tubular damage index
- TIMP-1 mRNA expression
- EGR1 mRNA expression
- EGR1 immunostaining

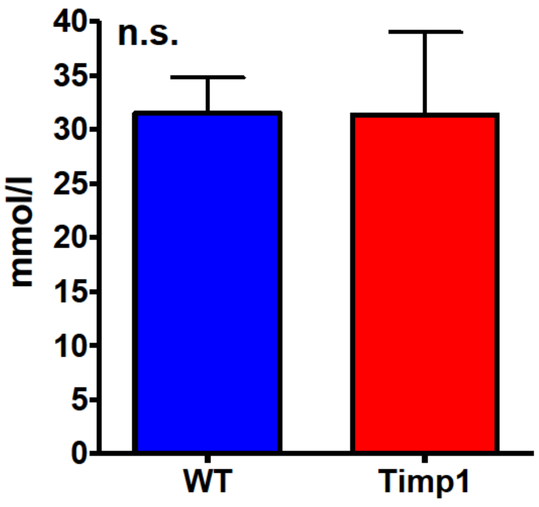
Statistics:

Data are presented as mean+SD. Student's t-test and Mann-Whitney test were performed (SPSS 10).

RESULTS

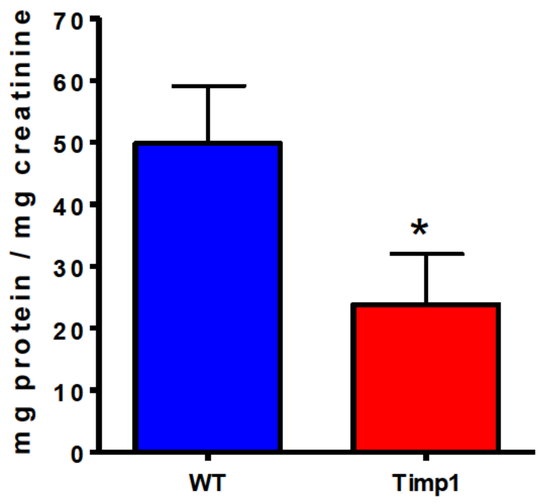
Serum glucose

Serum glucose levels similarly exceeded 30 mmol/l in both groups (n.s.).

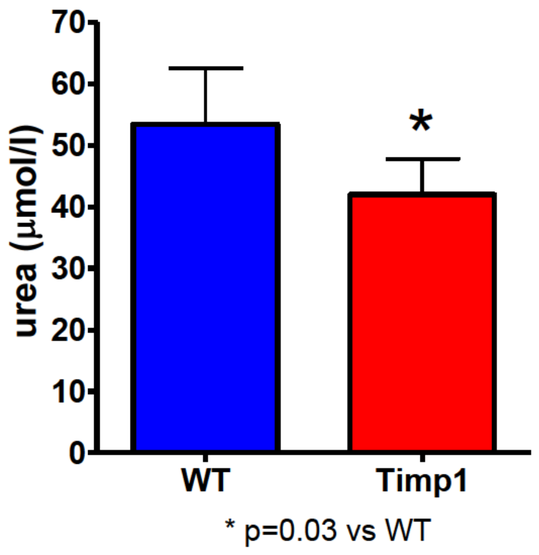


Renal function parameters

In TIMP-1 mice, urinary protein/creatinine ratio and serum urea level was reduced by 52% (p=0.0001) and by 21% (p=0.03), respectively.



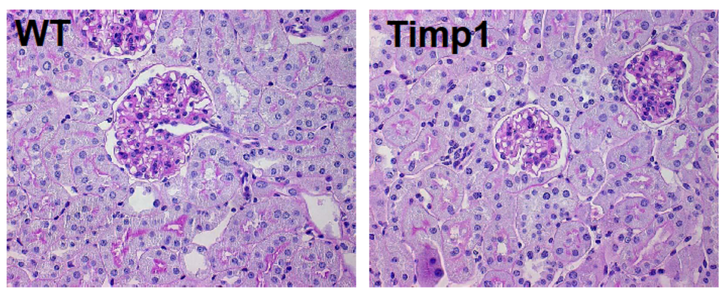
* p=0.0001 vs WT



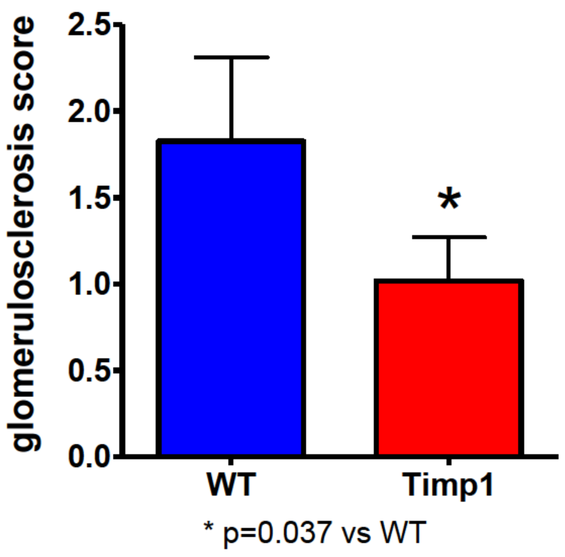
* p=0.03 vs WT

Histology

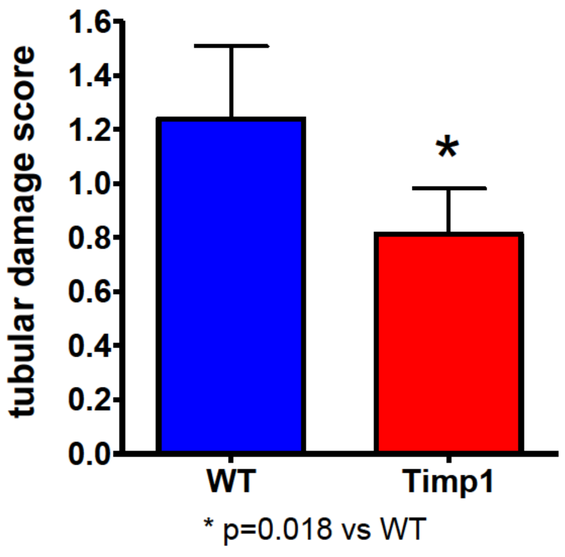
TIMP-1 mice depicted 44% lower score for glomerulosclerosis (p=0.037) and 34% less tubular damage (p=0.018) than WT kidneys.



PAS stain, 400x magnification



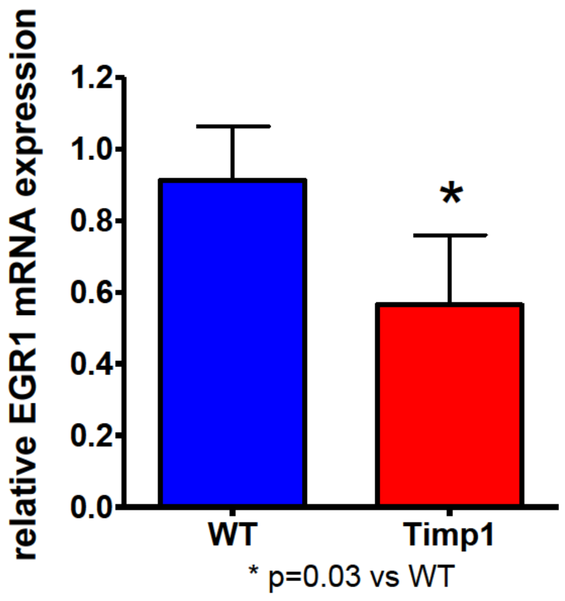
* p=0.037 vs WT



* p=0.018 vs WT

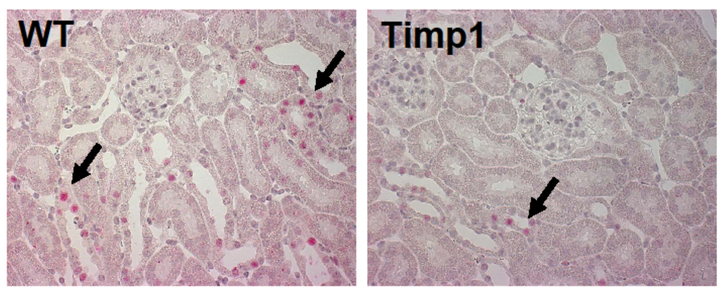
Renal EGR1 expression

In TIMP-1 mice, EGR1 mRNA expression was 42% lower (p=0.03) than in WT mice.

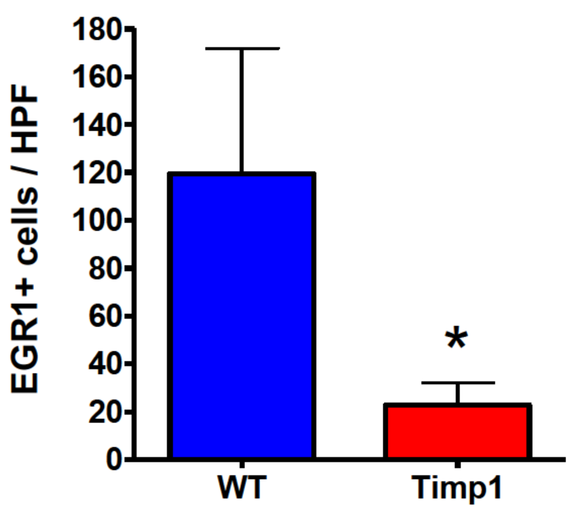


* p=0.03 vs WT

TIMP-1 kidneys depicted 60% decrease in EGR1 positive tubular cell number (see arrows below) as compared to WT (p=0.014).



EGR1 immunostaining (red), 400x magnification



* p=0.014 vs WT

CONCLUSION

Our results suggest that TIMP-1 not only contributes to diabetic nephropathy by direct inhibition of matrix degrading enzymes but also by its effect on the transcription factor EGR1, thus modulating fibroblast activation and proliferation.

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