The expression of full length MMP-2 and intracellular N-terminal truncated MMP-2 isoform in diabetic kidney disease

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Objectives:

Recently, matrix metalloproteinase-2 (MMP-2) has been regarded as a central of injury mechanism in heart and kidney. Especially, the ultrastructural examination of MMP-2 transgenic renal tubular epithelial cells demonstrated significant mitochondrial structural alterations and subsequent investigation lead to the discovery of a novel intracellular N-terminal truncated isoform of MMP-2 (NTT-MMP-2) generated by activation of an alternative intronic promoter The aim of this study is to explore the expression pattern and the role of full length MMP-2 and NTT-MMP2 (intracellular isoform) in diabetic kidney disease.

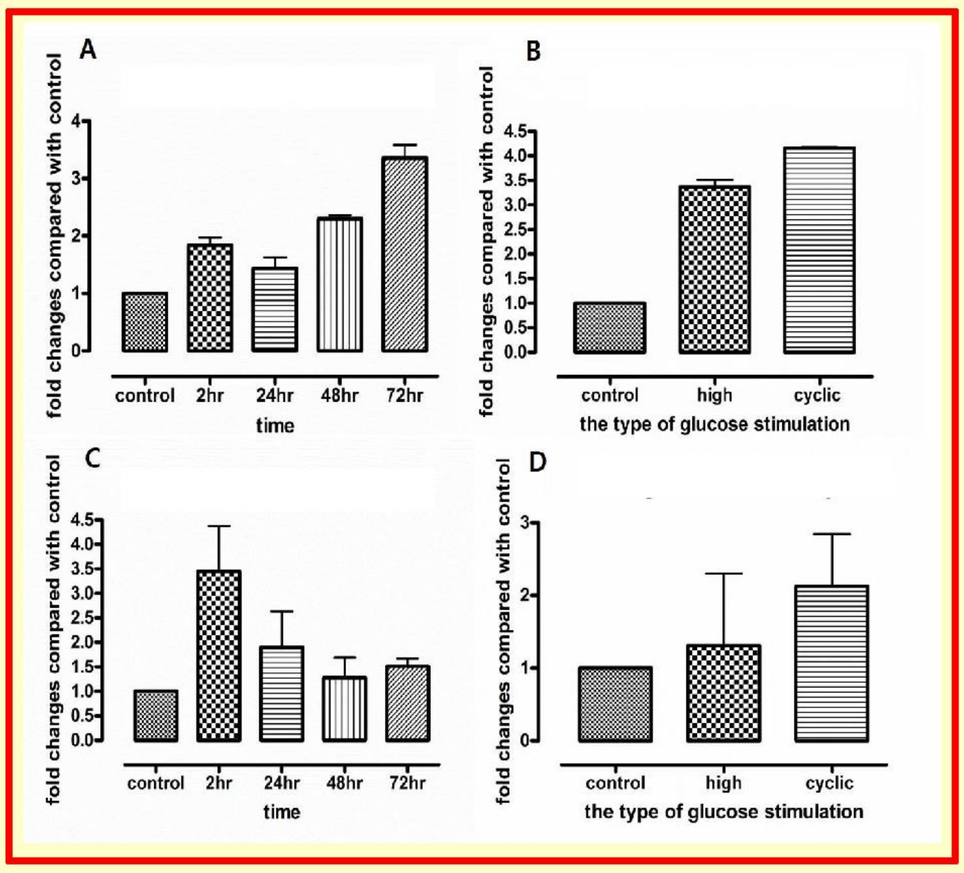
Methods:

In vitro study, we tested the glucose stimulation effects on HK2 cells. High, moderate, normal glucose were 30mM, 15mM, 5.6mM. Time dependent experiments for 2hr, 24hr, 48hr, 72hr were conducted and dose-dependent experiments for normal, high and cyclic (high-normal-moderate-normal) for the time of 48hr were performed. Also, real-time PCR was done for quantitative analysis of full length MMP-2 and NTT-MMP-2. In vivo study model using streptozotocin-induced diabetic mice, the expression of the full length MMP-2 and NTT-MMP-2 was assessed by immunohistochemistry using full length MMP-2 antibody (from Abcam Anti-MMP-2 antibody Catalog number 54401) and N-terminal truncated isoform specific affinity purified-goat-anti-NTT-MMP-2.

Results:

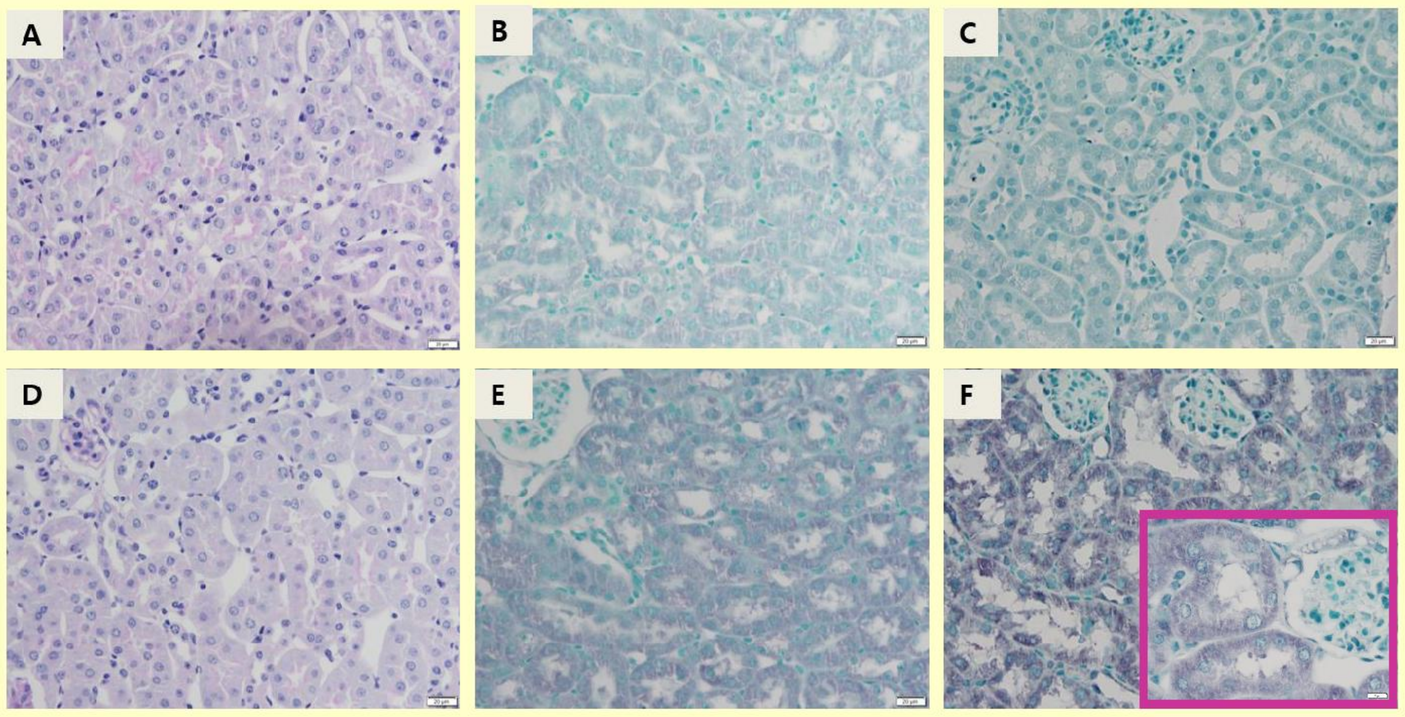
In vitro study using HK2 cells, high glucose stimulation induced the expression of FL-MMP-2 according to time and the expression at 72hr was higher by 3.4 times compared with control cells. Although the expression of NTT-MMP-2 was increased by high glucose, the peak expression time was different with FL-MMP-2 and the expression at 2hr was higher by 3.4 times compared with control cells. Also, the expression of NTT-MMP-2 was decreased according to time after 2hr stimulation. In dose-dependent experiments, cyclic stimulation induced higher expression of FL-MMP-2 and NTT-MMP-2 compared with control cells (FL-MMP-2; control vs. cyclic 1:4.16, NTT-MMP-2; control vs. cyclic 1:2.13). In streptozotocin-induced diabetic mice, the expression of FL-MMP-2 and NTT-MMP-2 was increased diffusely in the cytoplasm in renal tubular cell compared with normal control mice. In detail, FL-MMP-2 was expressed constitutively and the intensity or stained area were increased by induction of diabetes. However, NTT-MMP-2 was not expressed in normal control mice and was highly expressed after induction of diabetes mainly in renal proximal tubule.

<Glucose Stimulation Test: HK2 cell, qPCR data>



A, B: FL-MMP-2 / C, D: NTT-MMP-2
A, C: time-dependent experiment / B, D: experiment according to stimulation patterns

<Streptozotoxin-induced diabetic mice>



A, B, C: control mice / D, E, F: STZ-induced diabetic mice

A: In control group, brush borders of proximal tubules are well preserved on PAS stain (x400). **B**: Proximal tubules having intact brush border show weak positive (1+) immunoreactivity on full-length MMP-2 immunohistochemistry (x400). **C**: Negative for NTT MMP-2 immunohistochemistry (x400). **D**: Proximal tubules show diffusely denuded brush borders. Cytoplasmic vacuolation and epithelial cell loss are occasionally identified (x400). **E**: STZ-induced diabetic mice stained for FL-MMP-2. The expression of FL-MMP-2 was increased diffusely in the cytoplasm mainly in renal proximal tubular cells (x400). **F**: STZ-induced diabetic mice stained for NTT-MMP-2. The expression of NTT-MMP-2 was increased in the cytoplasm mainly in renal proximal tubular cells, not glomeruli and distal tubules (x400). NTT-MMP-2-stained patterns were different in comparison with FL-MMP-2, indicating that the expression of NTT-MMP-2 may be more localized and those could be related with mitochondria dysfunction (x1,000).

Conclusions:

Glucose stimulation induced the expression of full length MMP-2 and intracellular N-terminal truncated MMP-2 isoform in HK2 cell. Furthermore, experimental diabetes influenced on full-length MMP-2 expression and induced intracellular N-terminal truncated MMP-2 isoform in animal model.

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