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Two isoforms of Matrix Metalloproteinase-2 in diabetic nephropathy

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Background: Recent study uncovered the intracellular form called N-terminal truncated MMP-2 (NTT-MMP-2), which was generated by activation of an alternative intronic promoter unlikely full length MMP-2 (FL-MMP-2). The aim of this study is to explore the status of two isoforms of MMP-2 the expression in vitro, in vivo and human biopsy tissue, respectively and propose the new fundamental basis for diabetic nephropathy study related with MMP-2.

Methods: HK2 cells were cultured with different concentrations of D-glucose (5mM, 30mM) for 2, 24 and 48h, respectively and we also use 4-hydroxy-2-hexenal (HHE) as an another stimuli on HK2 cells. Two isoform of MMP-2 transcripts were measured using qPCR and the change of two isoforms of MMP-2 was checked after pyrrolidine dithiocarbamate (PDTC) treatment as an antioxidant and NF-kB inhibitor. Also, two isoforms of MMP-2 were tested in streptozotocin-induced diabetic mice at 12 weeks and 24 weeks, respectively. Finally, using human kidney biopsy tissue, the expressions of FL-MMP-2 and NTT-MMP-2 were analyzed by qPCR and immunohistochemical stain [controls (N=10) and diabetic kidney disease group (N=25)].

Results: Both FL-MMP-2 and NTT-MMP-2 transcripts significantly elevated by high glucose in HK2 cells. FL-MMP-2 was increased according to exposed time and has a peak expression by 15 times at 48h (15.4±0.7, p<0.001). Unlike FL-MMP-2, NTT-MMP-2 was more earlier peaked at 24h and approximately four times greater than control (3.9±0.2, p<0.001). By HHE stimulation, both FL-MMP-2 and NTT-MMP-2 transcripts also significantly upregulated in HK2 cell. PDTC inhibited NTT-MMP-2 transcription by high glucose, but FL-MMP-2 was not changed by PDTC. In STZ-induced diabetic mice model, FL-MMP-2 and NTT-MMP-2 transcripts were upregulated compared with control. Especially, NTT-MMP-2 expression was more strikingly increased compared with FL-MMP-2 in 24 weeks diabetic mice (7.2 fold change, p=0.003 vs. 1.8 fold change, p=0.002). NTT-MMP-2 was intensely increased in diabetic mice compared with control (p=0.001). NTT-MMP-2 was not stained at all in control mice. FL-MMP-2 and NTT-MMP-2 expression were found mainly within tubular epithelial cellular compartment, not glomeruli. Like animal model, two isoforms of MMP-2 were highly upregulated in human diabetic kidney disease. Specifically, FL-MMP-2 and NTT-MMP-2 transcripts were increased about by 12.5 and 3.1 times compared with control, respectively (p=0.001). Immunohistochemical staining grade was higher in diabetic kidney disease both in case of FL-MMP-2 and NTT-MMP-2, respectively (FL-MMP-2, 1.8±0.9 vs. 2.5±0.5, p=0.03; NTT-MMP-2, 0.9±0.7 vs. 2.4±0.6, p<0.001)

Conclusion: Two isoforms of MMP-2 is related with diabetic injury and NTT-MMP-2 is entirely induced by diabetes unlike FL-MMP-2. Further validated study is needed to support the pathogenic role of two isoforms of MMP-2 in diabetic nephropathy

Keywords: chronic kidney disease, Diabetic Nephropathy, matrix metalloproteinase-2, mitochondria