目的　通过siRNA（RNAi）干扰选择性下调TLR4基因的表达，探讨高糖对大鼠肾小管上皮细胞（NRK-52E）Toll样受体4（toll-like receptor 4，TLR4）及其转接子髓分化因子88（myeloid differentiation factor 88，MyD88）和炎性因子分泌的影响。 方法　设计并合成3对针对大鼠TLR4基因的特异性siRNA片段，以带有红色荧光的BLOCK-IT Alexa Fluor作为阴性对照，荧光显微镜下观察细胞转染效率，实时定量PCR检测TLR4 mRNA的表达变化。挑选基因沉默效率最佳的siRNA用于进一步实验，细胞被分5组：⑴ 正常糖对照组(NG)；⑵ 高糖组(HG)；⑶ HG+血管紧张素Ⅱ（AngⅡ）+空转组；⑷ HG+AngⅡ+siRNA组；⑸ HG+AngⅡ+厄贝沙坦（IrB）组。采用实时定量PCR法检测TLR4、MyD88 mRNA的表达，Western印迹检测TLR4、MyD88及核因子kappa B（NF-kB）蛋白表达；ELISA法检测巨噬细胞趋化蛋白1（MCP-1）、白细胞介素6（IL-6）的表达。 结果　与NG组比较，高糖组TLR4/MyD88 mRNA及TLR4、MyD88、NF-kB蛋白表达水平明显上调（均P＜0.01），细胞上清MCP-1、IL-6水平亦升高（P＜0.01）；空转组与高糖组比较差异无统计学意义（P＞0.05）； SiRNA组、ARB组TLR4、MyD88 mRNA明显下调，TLR4、MyD88及NF-kB蛋白明显下调， MCP-1、IL-6表达亦下调（均P＜0.01）。 结论　高糖上调小管上皮细胞TLR4/MyD88信号及炎性细胞因子表达，AngⅡ可增强此效应；特异性基因沉默可阻断由高糖及AngⅡ诱导的TLR4信号通路的激活，并下调炎性介质的释放；TLR4信号通路在高糖、高肾素环境肾小管上皮细胞炎性反应机制中发挥关键作用。
"/> Objective　To observe the expression of toll like receptor 4(TLR4) Signaling and the release of inflammation factors in rat tubular epithelial cell(NRK-52E) under high glucose condition after TLR4-siRNA transfection.　Methods Three TLR4-siRNA sequences were designed and synthesized. The transfection efficiency was observed by fluorescence microscope after transfection, and the expression of TLR4 mRNA was detected by real time PCR. The most effective siRNA was selected to be used for forward experiments. After transfection for 24 h, cells were stimulated with 25 mmol/L glucose and/or 10-7 mmol/L Angiotension Ⅱ(AngⅡ) for 12 h, 24 h; cells without stimulation were as normal control. Real-time PCR was used to analyze TLR4 and myeloid differentiation factor 88 (MyD88) mRNA expression; Western blot was used to observe TLR4/MyD88 and NF-κB protein expression. ELISA assay was used to detect the concentration of monocyte chemoattractant protein-1(MCP-1), interleukin-6(IL-6) in cell supernatant after cells were stimulated for 24 h.　Results　TLR4/MyD88 mRNA and TLR4/MyD88/NF-κB protein were highly expressed under high glucose or AngⅡ co-incubated NRK-52E(P＜0.01), the MCP-1 and IL-6 levels were also increased markedly compared with normal control group (P＜0.01). TLR4/MyD88 mRNA and TLR4/MyD88/NF-kB protein expressions were obviously inhibited in cells that were transfected with TLR4-siRNA compared with high glucose group(P＜0.01), MCP-1 and IL-6 production decreased remarkably compared with high glucose or　AngⅡ co-stimulated group(P＜0.01).　Conclusions　High glucose can lead to the activation of TLR4/MyD88/NF-kB signaling and the secretion of inflammation factors in NRK-52E,　AngⅡ further augments these effects. The effect can be blocked efficiently by specific siRNA gene silence. TLR4 signaling plays a pivotal role in the innate-immune inflammatory reaction in NRK-52E.
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SiRNA干扰对高糖环境下大鼠肾小管上皮细胞Toll样受体4/转接子髓分化因子88信号通路的影响

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Impact of TLR4-siRNA transfection on the expression of TLR4/MyD88 Signaling in rat under high glucose condition

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