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学位論文の内容の要旨

専　攻	分子情報制御医学	部　門	生体情報学
学籍番号	１４Ｄ７４８	氏　名	王　娟
論文題目	High glucose augments angiotensinogen in human renal proximal tubular cells through hepatocyte nuclear factor-5		

（論文要旨）

Background

Intrarenal renin-angiotensin system (RAS) plays a crucial role in development of diabetic nephropathy (DN), which is the major renal complication of diabetes mellitus as well as the main cause of end-stage renal disease. Previous studies reported that intrarenal angiotensinogen (AGT) mRNA and angiotensin (Ang) II levels were enhanced in type 2 diabetic rats. This could further lead to renal proximal tubular hypertrophy and interstitial fibrosis by stimulating reactive oxygen species and eventually result in diabetic nephropathy. Furthermore, by using human kidney biopsied samples, it has been demonstrated that AGT mRNA and protein levels in human renal cortex proximal tubules were significantly augmented in patients with type 2 diabetes. In the kidney, AGT is the only known substrate of renin as well as the rate-limiting factor for RAS activity, which is mainly expressed in renal proximal tubular cells (RPTCs). It has been manifested that AGT synthesis in RPTCs regulates intrarenal RAS activity for its contribution to renal accumulation of Ang II in rats. It has been demonstrated that high glucose treatment induced much higher levels of apoptosis and caspase-3 activity in rat RPTCs overexpressing AGT in comparison to control RPTCs in vitro, and induction of diabetes in transgenic mice that overexpressing AGT in RPTCs led to significant increases in apoptosis of RPTCs compared with diabetic nontransgenic littermates, moreover, the above effects were markedly attenuated by insulin and/or RAS blockers. Therefore, the level of AGT in RPTCs plays an important role in the pathogenesis of DN. In the case of rats, it has been reported that high glucose stimulated AGT synthesis in RPTCs and a glucose-responsive element located in the DNA sequences of AGT promoter has been identified. As to human, previous studies have succeeded in determining the region of human AGT promoter sequences. However, it is still unknown whether high glucose directly activates AGT synthesis in human RPTCs. In addition, the existence of glucose-responsive transcriptional factor(s) that bind(s) to AGT promoter sequences has not been identified. Therefore, the present study aimed to investigate the effects of high glucose on AGT in the RPTCs of human origin and identify the glucose-responsive transcriptional factor(s) that bind (s) to the DNA sequences of AGT promoter in human RPTCs.

Methods and Findings

Human kidney (HK)-2 cells were treated with normal glucose (5.5 mM) and high glucose (15.0 mM), respectively. Levels of AGT mRNA and AGT secretion of HK-2 cells were measured by real-time polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA), respectively. Consecutive 5'-end deletion mutant constructs and different site-directed mutagenesis products of human AGT promoter sequences were respectively transfected into HK-2 cells, followed by

AGT promoter activity measurement through dual luciferase assay. Three novel findings were uncovered in the present study. First, high glucose augments the levels of AGT mRNA and AGT secretion of human RPTCs. Second, high glucose augments AGT promoter activity in human RPTCs and the glucose-responsive region within human AGT promoter sequences is from -22 to -1,896. Third, HNF-5 is one of the glucose-responsive transcriptional factors which bind to the glucose-responsive region of AGT promoter sequences and mutation of its binding sites may abolish the high glucose effects on AGT in human RPTCs.

Discussion

Using GENETYX software and published data, candidates for glucose-responsive transcriptional factors were selected based on their likelihood of binding to human AGT promoter sequences. There may be other glucose-responsive transcriptional factors that work on AGT promoter, but we omitted them due to the lack of published supporting evidence for their potential involvement in high glucose-induced effects and/or AGT regulation. Therefore, other glucose-responsive transcriptional factors that bind to this region should be investigated further. In conclusion, the present study has demonstrated, for the first time, that high glucose augments AGT in human RPTCs through HNF-5, a glucose-responsive transcriptional factor that functions on the AGT gene promoter, which provides a potential therapeutic target for DN in humans.

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