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学位論文審査の結果の要旨

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論文題目	High glucose augments angiotensinogen in human renal proximal tubular cells through hepatocyte nuclear factor-5			
学位論文の審査結果	(合格) ・ 不合格　（該当するものを○で囲むこと。）			

〔 要 旨 〕

High glucose has been demonstrated to induce angiotensinogen (AGT) synthesis in the renal proximal tubular cells (RPTCs) of rats, which may further activate the intrarenal reninangiotensin system (RAS) and contribute to diabetic nephropathy. This 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. 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 mmunosorbent 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. High glucose significantly augmented the levels of AGT mRNA and AGT secretion of HK-2 cells, compared with normal glucose treatment. High glucose also significantly augmented AGT promoter activity in HK-2 cells transfected with the constructs of human AGT promoter sequences, compared with normal glucose treatment. Hepatocyte nuclear factor (HNF)-5 was found to be one of the glucose-responsive transcriptional factors of AGT in human RPTCs, since the mutation of its binding sites within AGT promoter sequences abolished the above effects of high glucose on AGT promoter activity as well as levels of AGT mRNA and its secretion. The present study has demonstrated, for the first time, that high glucose augments AGT in human RPTCs through HNF-5, which provides a potential therapeutic target for diabetic nephropathy.

Questions and Answers

Question 1: How's the distribution of HNF-5 in the whole body? Does HNF-5 express in other organs except kidney?

Answer 1: Except the kidney, HNF-5 is also reported to express in pancreas and liver.

Question 2: Have you detected the HNF-5 expression in proximal tubular cells?

Answer 2: We did not directly measure HNF-5 mRNA or protein in HK-2 cells. Fortunately, in the chromatin immunoprecipitation (CHIP) experiment, we successfully precipitated HNF-5 binding sequences by HNF-5 antibody from HK-2 cells. We believe this data indirectly suggests that HNF-5 does exist in proximal tubular cells.

Question 3: Does high glucose also increase AGT in other organs/tissues except kidney?

Answer 3: Yes, it has been demonstrated that high glucose also increases AGT in liver and adipose tissue.

Question 4: Do you think angiotensin Ⅱ receptor blocker (ARB) should affect the high glucose-induced AGT augmentation in proximal tubules or not?

Answer 4: Yes. In a previous study, it has been demonstrated that ARB significantly attenuates the augmented AGT in the proximal tubules of diabetic rats *in vivo*. However, in the present study, we found that valsartan, a kind of ARB, did not affect the high glucose-induced AGT augmentation of HK-2 cells *in vitro*. We think this should be due to the low renin activity and low AGT utilizing efficiency of HK-2 cell.

Question 5: Do you think ARB can affect liver AGT or not?

Answer 5: Yes, it has been demonstrated that candesartan, a kind of ARB, significantly decreases liver AGT.

Question 6: In general, what did you learn from your study?

Answer 6: First, I've learned how to come up with a new hypothesis or concept, based on the previous data. Second, I've learned how to design the study to prove our hypothesis. Third, in the whole process, I've learned several very important techniques and skills.

Question 7: How does high glucose activate HNF-5?

Answer 7: In fact, there is no definite answer as to this question from the published data. To my knowledge, the possible ways are as follows: 1. through regulating calcium influx; 2. through regulating reactive oxygen species (ROS); 3. through p38 mitogen-activated protein kinase (MAPK) pathway; 4. through PI3K/Akt pathway.

Question 8: How to up-regulate RAS in the liver?

Answer 8: To date, it has been demonstrated that liver injury, high glucose and glucocorticoid can up-regulate RAS in the liver?

Question 9: How to block HNF-5?

Answer 9: There are several possible ways: 1. Generate blocker/antibody to block the key sites of HNF-5; 2. Generate non-active competitive inhibitor that can bind to HNF-5 binding sites on AGT promoter sequences; 3. Inject antisense HNF-5 cDNA to inhibit HNF-5 expression.

Question 10: What are the other functions of HNF-5 in other organs except kidney?

Answer 10: HNF-5 also plays a pivotal role in the regulation of metabolism and in the differentiation of metabolic tissues such as the pancreas and liver. HNF-5 transcriptional factor binds to cis-regulatory elements in genes encoding gluconeogenic and glycolytic enzymes, serum proteins and hormones.

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