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学位論文審査の結果の要旨

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論文題目	Roles of Na+/H+ Exchanger Type 1 and Intracellular pH in Angiotensin II-Induced Reactive Oxygen Species Generation and Podocyte Apoptosis			
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 〕

Podocytes are terminally differentiated epithelial cells and play a key role both in maintenance of the glomerular filtration barrier and in glomerular structural integrity. Angiotensin II (Ang II) induces podocyte apoptosis both in vivo and in vitro. The reactive oxygen species (ROS), especially superoxide anion, play an important role in cell survival and apoptosis. Activation of Na^+/H^+ exchanger type 1 (NHE-1) and subsequent alkalinization of cytosol generally precedes the activation of many cellular functions, including apoptosis. It has been also reported that Ang II activates the NHE-1 in various tissues. NHE-1 predominately expresses in differentiated podocytes. In the present study, we hypothesized that Ang II induces podocyte apoptosis via NHE-1-induced pHi changes and NADPH oxidase-derived ROS production.

We examined the effects of Ang II (100 nmol/L) on apoptosis, ROS production, and intracellular pH (pHi) in podocytes. For intracellular ROS and pH measurements, image analysis was conducted using confocal laser microscopy after incubation with DHE and SNARF-1 staining. ROS and pHi were elevated with Ang II treatment. Apoptotic cell numbers, as measured by TUNEL staining and caspase 3 activity, were also augmented in the Ang II–treated group. Pre-treatment with RNH-6270 [an active form of olmesartan medoxomil, 100 nmol/L, an Ang II type 1–receptor (AT1R) blocker], apocynin (50 μmol/L, NADPH oxidase inhibitor), or 5-N, N-hexamethylene amiloride (HMA, 30 μmol/L, NHE-1inhibitor) abolished Ang II–induced podocyte apoptosis, whereas NHE-1 mRNA and protein expression was not affected by Ang II treatment. Moreover, Ang II increased NHE-1 phosphorylation

These results suggest that ROS production, NHE-1 activation, and intracellular alkalization were early features prior to apoptosis in Ang II–treated mouse podocytes, and may offer new insights into the mechanisms responsible for Ang II–induced podocyte injury.

上記発表に対し、以下の質疑応答が適切におこなわれた。

【質問1】 Have you examine dose-dependent effects of Ang II on podocytes apoptosis?

応答: Preliminary studies examined dose-depend effects of Ang II (0.1, 1, 10, 100 nM) on ROS generation and pHi changes. Data showed that after 30 min administration of Ang II, ROS generation

and pHi were significantly increased at 10, 100 nM and 100 nM, respectively. Previously studies (Am J Med Sci. 2012) have also shown that Ang II induced podocytes apoptosis in a dose-dependent (10^{-12} to 10^{-6} M) manner.

【質問 2】 Why can you conclude that Ang II induces the ROS production through AT1R-dependent pathway? Did you use AT2R antagonist?

応答: Although we have not examined the effects of an AT2R antagonist in the present study, we have shown that Ang II stimulates ROS formation though AT1 receptor in other cells.

【質問3】 What about the effects of AT1R siRNA on ROS production?

応答: In our experiment, Ang II-induced the ROS generation could inhibit by an AT1R antagonist. Since AT1R antagonist has highly sensitivity to AT1R, we didn't use AT1R siRNA in this study.

【質問 4】 What is the mechanism responsible for pHi-induced apoptosis?

応答: Several reports have suggested that NHE-1 promotes apoptosis via actively inducing intracellular alkalinization, activation of Bax (Proc Natl Acad Sci USA.1999), the inhibition of mitochondrial ADP transport (Biol Chem. 2001), and endonucleases (Semin Immunol.1992). However, the mechanisms behind alkalinization related apoptosis still need to be evaluated.

【質問 5】 Why did you choose the 14-3-3 phosphorylation site in NHE-1?

応答: Previously paper (Circ Res. 1997) has shown that Ang II increased pHi via NHE-1 activation by phosphorylation the 14-3-3 site of NHE-1. However, others phosphorylation sites should also be investigated in the future.

【質問 6】 Did you check the expression of NADPH oxidase subunits?

応答: We didn't check the expression of NADPH oxidase subunits in this experiment, because it has already been shown that Ang II-dependent increases in NADPH oxidase activity and subunit expression (NOX2, NOX4, Rac1, and p22phox) and ROS generation in cultured podocytes (Hypertension. 2008).

【質問 7】 Did you examine ROS generator paraquat in the experiment?

応答: We have not examined the effects of ROS generator paraquat in this experiment. Since paraquat induces apoptosis in many cell lines and NHE-1 is a known target for ROS (Pflugers Arch. 2011), paraquat might activate the NHE-1 and result in podocyte apoptosis.

【質問 8】 Did you know any other substances that could induce podocyte apoptosis?

応答: There are many substances that could cause podocyte apoptosis, like high glucose, advanced glycation endproducts, TGFβ1, aldosterone, etc.

【質問 9】 Why did you focus on the podocyte, not on the other component of glomerular filtration barrier?

応答: Podocytes have a predominant role in maintaining the integrity of the glomerular filter: they are the primary source of the GBM components laminin β2 (Development. 2006) and the collagen α3α4α5 (IV) network (J. Am. Soc. Nephrol. 2009), they initiate fenestrate formation in endothelial cells, they secrete the proangiogenic factors vascular endothelial growth factor-A and angiopoetin-1 (J. Am. Soc. Nephrol.2002, 2004). Thus, podocyte injury is now clearly recognized as a major cause of albuminuria.

【質問 10】 What about the effect of its inhibitor on other organs?

応答: NHE-1 is a ubiquitously expressed integral membrane protein, which regulates intracellular pH in mammalian cells. In this experiment, only treated podocytes with NHE-1 inhibitor for 18h had no effect on podocytes apoptosis. In the clinic and animal experiments, the NHE-1 inhibitor mostly treated for the acute injury (heart and brain). Future studies should be needed to investigate the long effect of NHE-1 inhibitor.

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