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様式１０７

学位論文審査の結果の要旨

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論文題目	A protease-activated receptor-1 antagonist protects against podocyte injury in a mouse model of nephropathy			
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 〕 The kidney expresses protease-activated receptor-1 (PAR-1). PAR-1 is known as a thrombin receptor, but its role in kidney injury is not well understood. We examined the contribution of PAR-1 to kidney glomerular injury and the effects of its inhibition on development of nephropathy. Mice were divided into 3 groups: control, doxorubicin +vehicle and doxorubicin +Q94 (PAR-1 antagonist Q94) groups. Where indicated, doxorubicin was administered intravenously and PAR-1 antagonist or saline vehicle by subcutaneous osmotic mini-pump. PAR-1 expression was increased in glomeruli of mice treated with doxorubicin. Q94 treatment significantly suppressed the increased albuminuria in these nephropathic mice. Pathological analysis showed that Q94 treatment significantly attenuated periodic acid-Schiff and desmin staining, indicators of podocyte injury, and also decreased glomerular levels of podocin and nephrin. Furthermore, thrombin increased intracellular calcium levels in podocytes. This increase was suppressed by Q94 and Rox4560, a transient receptor potential cation channel (TRPC)3/6 antagonist. In addition, both Q94 and Rox4560 suppressed the doxorubicin-induced increase in activities of caspase-9 and caspase-3 in podocytes. These data suggested that PAR-1 contributes to development of podocyte and glomerular injury and that PAR-1 antagonists have therapeutic potential.

[質問 1]Can you explain why PAR-1 and TRPC3/6 induced apoptosis via a shared mechanism?

応答：Caspase-3 and 9 which are known to induce apoptosis, in cultured podocytes. Q94 suppressed the doxorubicin-induced increase in activities of both caspase-3 and 9. Rox4560 also suppressed the increase

in activities of both caspase-3 and 9. In addition, doxorubicin treated podocytes were also administered Q94 (60 μM) and Rox4560 (1.2 μM) together. No further decrease in caspase-9 activity was observed, compared with the cells treated with only Q94 or Rox4560. This suggested that PAR-1 and TRPC3/6 induced apoptosis via a shared mechanism.

[質問 2]

Why in the control group only have 4 mice, and only 4 is sufficient for statistical analysis?

応答：When we design this experiment, there are 6 mice in control group. Unfortunately 2 mice died because of accident. In case of something happen during experiment I should increase the mouse number in the future study. We use One-way ANOVA(and nonparametric)to analysis the data.

[質問 3]

Why you use thrombin to stimulate the calcium influx but doxorubicin to induce apoptosis in podocytes?

応答：In the preliminary experiment, we also try to use thrombin to induce apoptosis. But only the thrombin cannot induce apoptosis, I thought we should add other risk factors. Such as high glucose condition. On the other hand, we didn't use doxorubicin to induce calcium response. Because doxorubicin as a renal toxicity compound, the specific role not so clear. If we use it, we cannot confirm the calcium response was induced by PAR-1 signaling.

[質問 4]

PAR-1 was also express in endothelium cell. Why you can say PAR-1 play very important role only in podocyte?

応答：Yes, PAR-1 also express in podocyte. We measure the mRNA of nephrin and podcin, which only express in podocyte. The loss of both nephrin and podocin mRNA levels was attenuated by Q94.

[質問 5]

Why you use C57bl/6 mouse for anti-GBM but use Balb/c for doxorubicin?

応答：In the preliminary experiment, we also use C57bl/6 for doxorubicin. But this kind of mouse was resist to doxorubicin. In order to confirm the result we ask some anti-GBM sample from cooperator.

[質問 6]

Why is PAR-1 increased in the diseased kidney?

応答：This is a very complicated question, at least according to this study, by overloading calcium the PAR-1 increased in the diseased kidney.

[質問 7]

How did PAR-1 related with TRPC3/6? What is the shared mechanism?

応答：PAR-1 was a G protein couple receptor. Binding of an agonist to a Gq-protein-coupled receptor leads to phospholipase C (PLC) activation. The activated PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2] to produce of diacylglycerol (DAG) and IP3. DAG and the reduction of PI(4,5)P2 levels directly contribute to TRPC channels activation, while IP3 triggers Ca2+ release from intracellular stores.

[質問 8]

What is the specificity of Q94 on PAR-1? Where is Q94 binding in PAR-1?

応答：PAR1 negative allosteric modulator; inhibits PAR1-Gαq interaction. Selective for PAR1 over PAR2. Blocks thrombin-induced intracellular calcium mobilization.

[質問 9]

Is Q94 available for DMN patients?

応答：So far, this compound only used in basic research field. For example the side effect still need research in the future study.

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