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研究課題名(和文) Effects of neutralizing (pro)renin receptor antibody on the podocytes injury in diabetic nephropathy

研究課題名(英文) Effects of neutralizing (pro)renin receptor antibody on the podocytes injury in diabetic nephropathy

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研究成果の概要(和文)：高いブドウ糖干渉が浸透圧対照群と比較してnephrinのmRNA表現力を減らすことを、遺伝子発現データは明らかにしました、しかし、遺伝子発現のレベルは非常に少なかったです。したがって、(P)RR mAbにはpodocytesを有害な影響から保護する可能性があるという我々の仮説と、podocinデータは一致しています。

研究成果の学術的意義や社会的意義

The podocin gene expression data suggests the protective effects of (P)RR mAb on high glucose-induced podocyte injury, therefore the study will link a translational approach to clinical benefits.

研究成果の概要(英文)：In vitro studies were conducted to determine the protective effects of (P)RR monoclonal antibodies (mAb) in podocyte injury. The gene expression data revealed that high glucose intervention reduced the mRNA expression of nephrin compared to the osmotic control group, although the level of gene expression was very small. In contrary, (P)RR mAb treatment caused the increase expression of nephrin mRNA, suggestive of the reduced podocyte injury. In case of podocin and synaptopodin the expression level is unsatisfactory. Therefore, we tried to establish the podocyte injury model in the human immortalized podocyte, AB 8/13 cells. Gene expression of podocin, nephrin and synaptopodin in this human podocyte cell line were also not satisfactory. Therefore, the podocin data are in line with our hypothesis that (P)RR mAb has the potential to protect the podocytes against injurious effects.

研究分野：Kidney Injury

キーワード：Podocyte injury Diabetic nephropathy (P)RR antibody High glucose

様式 C - 19、F - 19 - 1、Z - 19、CK - 19 (共通)

## 1 . 研究開始当初の背景

Podocytes are highly specialized, terminally differentiated epithelial cells located on the surface of the capillaries of glomeruli, and normally prevent leakage of protein into the urine [Zhou L and Liu Y, *Nat Rev Nephrol.* 2015; 11(9):535-45]. Since podocytes are unable to divide, their injury and malfunction leads to proteinuria, accumulation of extracellular matrix, and glomerulosclerosis [Zhou L and Liu Y, *Nat Rev Nephrol.* 2015; 11(9):535-45]. Therefore, podocyte injury is a major contributor of diabetic nephropathy which often leads to end-stage renal disease. Although (P)RR is considered as an integral component of renin-angiotensin-aldosterone system (RAAS), however, this receptor has several RAAS independent functions especially in the cell transduction pathway [Nabi A.H.M. N and Suzuki F, *Protein Interaction*, chapter 13, 243-274]. (P)RR is critical for podocytes because this receptor is essential for autophagy and survival [Riediger F et al. *J Am Soc Nephrol.* 2011; 22(12):2193-202]. In contrast, (P)RR is up-regulated in the diabetic kidney podocytes which exposed to high glucose [Nabi A.H.M. N and Suzuki F, *Protein Interaction*, chapter 13, 243-274]. Up-regulation of (P)RR generates intracellular signal molecules, such as phosphorylation of ERK1/2 and p38, leading to inflammation and matrix formation. Down-regulation of PRR expression reversed high glucose-induced inflammation, implying that (P)RR may contribute to the pathophysiology of diabetic kidney disease [Huang J and Siragy HM. *Endocrinology.* 2009; 150: 5557–5565; 6,7; Huang Y et al. *Kidney Int.* 2006; 69: 105–113.]. Canonical Wnt- $\beta$ -catenin signaling pathway component expression is increased in glomeruli and podocytes of hyperglycemic patients and mouse model of diabetic kidney disease and plays a critical role in integrating cell adhesion, motility, cell death, and differentiation [Kato H et al. *J Biol Chem.* 2011; 286: 26003–26015]. Recently, PRR was found to be an accessory subunit for V-ATPase, which contributes to the activation of the canonical Wnt- $\beta$ -catenin signaling pathway [Kato H et al. *J Biol Chem.* 2011; 286: 26003–26015]. Interestingly, it is also demonstrated that high glucose-induced podocyte injury is mediated by activated PRR-Wnt- $\beta$ -catenin signaling pathway. Importantly, downregulation of PRR expression attenuated high glucose-induced Wnt- $\beta$ -catenin signaling pathway and improved podocytes structural and functional abnormalities [Li C and Siragy HM. *PLoS One.* 2014; 9(2):e89233]. However, translational therapeutic approach to address this issue is remained to be elucidated.

## 2 . 研究の目的

From our ongoing studies, we have demonstrated several pieces of evidences that *de novo* neutralizing (P)RR antibody has the potential to block the  $\beta$ -catenin signaling pathway in pancreatic ductal adenocarcinoma cells. Therefore, this study will be conducted to explore the protective effects of *de novo* neutralizing (P)RR antibody on podocyte injury as well as diabetic nephropathy.

## 3 . 研究の方法

Conditionally immortalized mouse podocytes were cultured on collagen I coated plates in RPMI 1640 medium containing 10% fetal bovine serum supplemented with 100 U/mL of recombinant mouse interferon- $\gamma$  (INF- $\gamma$ ) at 330C, which is considered as growth-permissive conditions. To differentiate the podocytes, cells were placed at 37<sup>o</sup>C for 14 days without INF- $\gamma$ , and subsequently, these differentiated cells were used for generating high glucose-induced podocyte injury model. The differentiated podocytes were plated on 24-well plate and after reaching at 70-80% confluence, cells were serum starved for overnight. After differentiation, podocytes were cultured for 72 hours in medium containing 5 mM D-glucose plus 20 mM D-mannitol (osmotic control); 25 mM D-glucose (high glucose); and 25 mM

D-glucose (high glucose) with 200 microgram/mL (P)RR neutralizing mAb (clone 48\_8). Following intervention, cells were lysed and homogenized in trisol solution, and extraction of RNA as well as preparation of cDNA were performed in accordance with our established protocol. Finally, quantitative real time PCR was performed for these cDNA to check the mRNA expression of podocyte injury markers,; nephrin, podocin and synaptopodin.

#### 4 . 研究成果

The gene expression data revealed that high glucose intervention reduced the mRNA expression of nephrin compared to the osmotic control group, although the level of gene expression was very small. In contrary, (P)RR mAb treatment caused the increase expression of nephrin mRNA, suggestive of the reduced podocyte injury (Fig.1). In case of podocin and synaptopodin the expression level was unsatisfactory. Therefore, we tried to establish the podocyte injury model in the human immortalized podocyte, AB 8/13 cells. Gene expression of podocin, nephrin and synaptopodin in this human podocyte cell line were also not satisfactory. Therefore, it is hard to conduct other experiments in the above mentioned podocyte injury model to test the effects of our neutralizing (P)RR mAb in detail. However, the podocin data are in line with our hypothesis that (P)RR mAb has the potential to protect the podocytes against injurious effects.

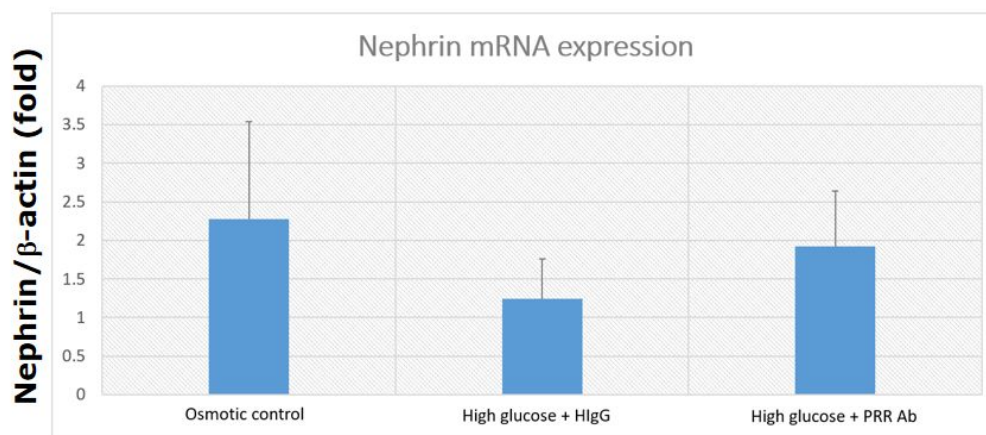


Fig 1: mRNA expression of Nephrin

#### 5 . 主な発表論文等

〔雑誌論文〕(計 2 件)

1. Asadur Rahman, Arif Ul Hasan, Akira Nishiyama and Hiroyuki Kobori. Altered Circadian Timing System-Mediated Non-Dipping Pattern of Blood Pressure and Associated Cardiovascular Disorders in Metabolic and Kidney Diseases. *International Journal of Molecular Sciences*. Vol. 19, 2018, 1-21. 10.3390/ijms19020400.
2. Asadur Rahman, Arif Ul Hasan, Hiroyuki Kobori. Melatonin in chronic kidney disease: a promising chronotherapy targeting the intrarenal renin angiotensin system. Vol. 42, No. 6. 2019, 920-923. doi: 10.1038/s41440-019-0223-9.

〔学会発表〕(計 2 件)

1. Asadur Rahman, Yuki Shibayama, Arif Ul Hasan, Hirofumi Hitomi, Daisuke Nakano, Hiroyuki Kobori, Akira Nishiyama. Antiproliferative effects of polyclonal antibody against (pro)renin receptor in pancreatic ductal adenocarcinoma cells. 18th World Congress of Basic and Clinical

Pharmacology. 2018.

2. Asadur Rahman, Arif Ul Hasan, Katsuya Miyake, Akira Nishiyama and Hiroyuki Kobori. Neutralization of (pro)renin receptor and inhibition of autophagy on the metastasis of breast carcinoma cells. 第8回 国際医療福祉大学学会学術大会スケジュール(国際学会). 2018

〔図書〕(計 0 件)

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権利者：  
種類：  
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〔その他〕

ホームページ等

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