Diabetic Complications Consortium

Application Title: Protease-activated receptors in diabetic complications

Principal Investigator: Nobuyuki Takahashi

1. Project Accomplishments:

Our aims of this application are 1) to clarify whether inhibiting both PAR1 and PAR2 is more effective in preventing diabetic kidney disease (DKD) than inhibiting PAR2 alone, and 2) to obtain seed/seeds of PAR2 antagonist. Our results show that pro-inflammatory effect of PAR1 in HUVEC was much larger than that of PAR2. But this could be because the expression level of PAR1 is more than 20 times higher than that of PAR2 in HUVEC. The *in vivo* experiment to answer the same question will be summarized shortly. We successfully identified 2 seeds of PAR2 antagonists from small molecule library containing 6500 chemical compounds produced in Tohoku University Graduate School of Pharmaceutical Sciences.

2. Specific Aims:

Specific Aim 1 will clarify whether inhibiting both PAR1 and PAR2 is more effective than inhibiting PAR2 alone in preventing DKD.

Results:

We tested the effect on *MCP1* and *PAI1* expression of inhibiting PAR1, PAR2, or both using HUVEC (4.5 g/L glucose). Human FXa increased the level of *MCP1* expression, which was reduced by a PAR1 inhibitor (E5555). But a PAR2 inhibitor (FSLLRY) did not affect *MCP1* expression level, and did not further reduce *MCP1* level by E5555 (Figure 1A). Increased level of *PAI1* expression by FXa was reduced only by inhibiting both PAR1 and PAR2 (Figure 1B).

The results indicate that inhibiting the

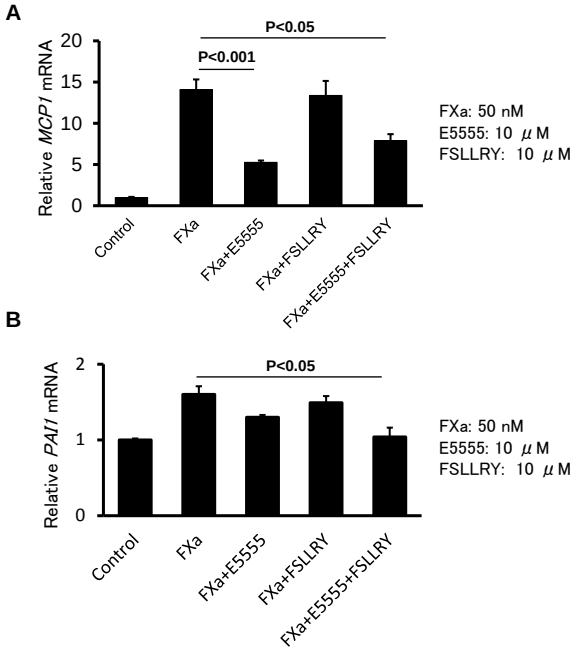

Fig. 1 Expression levels of inflammatory genes by inhibiting PAR2, PAR1, or both

expression of pro-inflammatory cytokines by PAR2 antagonist and PAR1 antagonist could be additive, but the effect of PAR1 inhibitor was much greater than that of PAR2 in our experimental condition. This could be because the expression level of *PAR1* is more than 20 times higher than that of PAR2. Because the results may not recapitulate diabetic complications *in vivo*, evaluating the effect of PAR2 and PAR1 *in vivo* is necessary.

Accordingly, we have been generating *Ins2*$^{Akita/+}$; *Nos3*$^{-/-}$ as a mouse model of DKD as we previously described (Wang CH, Takahashi N. et al. *PNAS* 2011; 108:2070-2075). These mice were divided into four groups and treated with PAR2 inhibitor (FSLLRY, 4 mg/kg/day), PAR1 inhibitor (E5555, 50 mg/kg/day), or both, beginning at the age of 16 weeks for 4 weeks. The conclusion from this study will be summarized shortly.

We also clarified that lack of PAR2 is useful to treat adenine-induced CKD mice (Hayashi et al. BBRC 2017), suggesting that antagonizing PAR2 is useful to treat CKD other than DKD. Diabetes with other CKD could deteriorate DKD.

Specific Aim 2 is to identify chemical compounds potentially useful as PAR2 antagonist(s).

Results: We screened small molecule library composed of approximately 6500 chemical compounds that were produced in Tohoku University Graduate School of Pharmaceutical Sciences. We first screened compounds that inhibited 50% or more of the increase in intracellular Ca signal of human endothelial cell line (EA.hy926) by PAR2 agonist (2f-LIGRLO, 20 μM) as shown in Figure 2A. We identified 116 compounds in this screening. Next we screened these 116 compounds and identified 2 seeds that prevented 50% or more of the increase in the expression of *MCP1* by PAR2 agonist (Figure 2B).

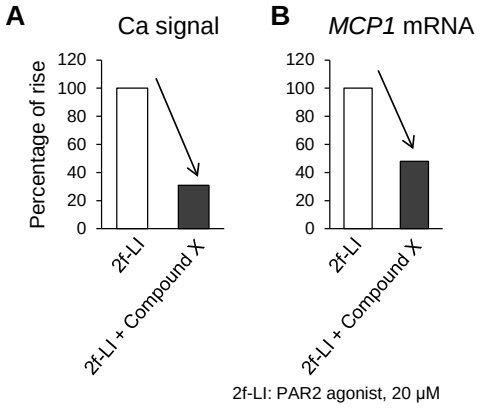

Fig. 2 Screening of novel PAR2 inhibitor(s)

3. Publication:

Hayashi S, Oe Y, Fushima T, Sato E, Sato H, Ito S, Takahashi N. Protease-activated receptor 2 exacerbates adenine-induced renal tubulointerstitial injury in mice. **Biochem Biophys Res Commun** 2017 Jan 29; 483(1):547-552.