学位論文（要約）

Development of novel therapeutic agents for refractory sight-threatening ocular diseases

（ 難治性眼疾患に対する新規治療薬の開発 ）

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【Background and Objectives】

Neovascular age-related macular degeneration (nAMD) and uveitis are two representative refractory sight-threatening diseases with long-term continuous therapy, high rate of recurrence, and disappointing prognosis in some of the patients. Therefore, it is necessary to develop new therapies for patients suffering from these diseases. Our group has previously identified (pro)renin receptor [(P)RR] as a pivotal molecule involved in the pathogenesis of ocular inflammation and angiogenesis. Most recently, we developed a novel single-stranded RNA interference (RNAi) agent against (P)RR [(P)RR-PshRNA] and confirmed its suppressive effect in ocular inflammation models in mice. In this study, we investigated the therapeutic effect of (P)RR-PshRNA on CNV and subretinal fibrosis, together with the underlying molecular mechanisms. In addition, platelet-derived growth factor (PDGF) signaling was previously revealed to be associated with angiogenesis and fibrosis formation in several organs, but it is still unknown whether it is involved in subretinal fibrosis or not. Therefore, we also examined the effect of platelet-derived growth factor receptor-β (PDGFR-β) blockade on CNV and subretinal fibrosis. Furthermore, our group has previously revealed that inhibition of the nuclear factor-κB (NF-κB) signaling ameliorated experimental autoimmune uveoretinitis (EAU) model in mice, which is a widely-used model for endogenous uveitis in human. In the current study, we also evaluated the therapeutic effect of IMD-0354, a specific non-ATP binding IκB kinase (IKK)β inhibitor, on EAU in mice.

【Methods】

Laser-induced CNV model was generated in C57BL/6J mice and retinal pigment epithelium (RPE)-specific (P)RR-CKO mice, respectively. Drugs or controls were injected into the vitreous cavity immediately after laser injury. Real-time qPCR was performed to check the expression of inflammatory and fibrotic molecules at 3 days after laser injury. The size of CNV (stained by Isolectin-B4) and subretinal fibrosis (stained by anti-type I collagen antibody), macrophage infiltration (stained by F4/80 antibody), and subretinal hyperreflective material (SHRM) were quantified,

respectively. Immunoblot analyses were conducted to examine the phosphorylation of ERK1/2 and SMAD2. Surgically removed tissues from nAMD patients were used to confirm the localization of (P)RR. EAU model was induced in B10.BR mice. Clinical and histopathological severities were graded. The translocation of NF-κB p65 into the nucleus of retina was assessed. T cells were collected from draining lymph nodes to examine inflammatory cytokine production and T-cell proliferation.

【Results】

(1) Administration of (P)RR-PshRNA significantly suppressed CNV formation, the expression of CNV-related inflammatory molecules via inhibition of phosphorylation of the ERK1/2 pathway, and macrophage infiltration in the CNV mouse model. (P)RR-PshRNA also downregulated the expression of *Tgfb1*, thereby suppressing subretinal fibrosis formation together with EMT-related markers and phosphorylated SMAD2 expressions. RPE-specific gene ablation of (P)RR also ameliorated CNV and subretinal fibrosis. Furthermore, the therapeutic effect of (P)RR-PshRNA was comparable to that of aflibercept, one of the first-line drugs for nAMD in clinical practice. Importantly, nAMD patient specimens demonstrated the co-localization of (P)RR with phosphorylated ERK1/2 in neovascular endothelial cells and RPE cells.

(2) Blockade of PDGFRβ by intravitreal injection of PDGFRβ neutralizing antibody significantly suppressed CNV and subretinal fibrosis, and the size of SHRM, which is a morphological feature indicating subretinal fibrosis in nAMD patients. Mechanistically, the blockade of PDGFRβ significantly prevented the recruitment of pericytes to the CNV lesions. Notably, the blockade of PDGFRβ suppressed the pericyte migration induced by PDGF-BB in a dose-dependent manner.

(3) IMD-0354 significantly ameliorated pathological changes in EAU mice via inhibition of the NF-κB p65 translocation into the nucleus EAU retinas. The production of uveitis-related inflammatory cytokines including *TNF-α* (tumor necrosis factor-α), *IFN-γ* (interferon-γ), *IL-1α* (interleukin-1α), *IL-*

6, *IL-17A*, all of which are Th1 and Th17-mediated molecules, were also inhibited by IMD-0354 *in vitro*.

【Discussion】

The present study, for the first time, provides several new findings on the development of novel therapies for nAMD and uveitis. In the current study, we expanded the application of (P)RR-PshRNA to nAMD, revealing that inhibition of (P)RR and its downstream signaling could ameliorate the development of both CNV and subretinal fibrosis, together with the underlying molecular mechanisms. Besides, although previous studies revealed that PDGFRβ signaling participated in the process of angiogenesis and fibrosis via regulation of pericytes in several organs including the liver, kidney, and spinal cord, it is still unknown whether PDGFRβ signaling contributed to the formation of subretinal fibrosis via regulation of pericytes. Our study revealed, for the first time, PDGFRβ and pericytes were associated with subretinal fibrosis. Inhibition of PDGFRβ signaling could prevent the recruitment of pericytes and therefore suppress fibrosis formation, suggesting that pericytes, apart from vascular endothelial cells, might be a promising therapeutic target for the treatment of nAMD in clinical practice. Further studies are still ongoing to investigate the underlying molecular mechanisms. Furthermore, we also demonstrated the IKKβ might play a pivotal role in ocular inflammation via its regulation of NF-κB translocation into the nucleus and the subsequent expression of proinflammatory molecules. Selective inhibition IKKβ with IMD-0354 led to significant suppression of inflammatory responses in EAU model, suggesting that IMD-0354 is a promising therapeutic agent for endogenous uveitis.

Conclusion:

Our current study provides solid evidence for the development of novel therapies for nAMD and uveitis, both of which are refractory sight-threating diseases.