# 学位論文内容の要旨
# （Summary of Dissertation）

博士の専攻分野の名称　博士（医　学）　氏 名　劉　野
（Degree conferred: Doctor of Philosophy）　（Name　LIU Ye）

## 学 位 論 文 題 名
## （Dissertation Title）

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法の開発 ）

【Background and Objectives】Neovascular age-related macular degeneration (nAMD) and uveitis are two representative refractory sight-threatening diseases with a long-term continuous therapy and follow-up period, high rate of recurrence and disappointing prognosis with currently standard therapy in some of the patients. Therefore, it is necessary to develop new therapies for those patients suffering from these diseases. Our group have previously identified (pro)renin receptor [(P)RR] as a pivotal molecule contributing to the pathogenesis of ocular inflammation and angiogenesis. We recently 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. 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. In current study, we aimed to investigate the effect of (P)RR-PshRNA and platelet-derived growth factor receptor (PDGFR)-β blockade on choroidal neovascularization (CNV) and fibrosis. In terms of uveitis, we have previous revealed that inhibition of the nuclear factor-κB (NF-κB) signaling ameliorated experimental autoimmune uveoretinitis (EAU). In this study, we also aimed to investigate the effect of IMD-0354, a specific non-ATP binding IκB kinase (IKK)β inhibitor on experimental autoimmune uveoretinitis in mice, together with the underlying molecular mechanisms, respectively.

【Methods】Laser-induced CNV model was induced in C57BL/6J mice and retinal pigment epithelium (RPE)-specific (P)RR/Atp6ap2-cKO mice. Drugs and the 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 including epithelial-mesenchymal transition (EMT)-related transcriptional factors at 3 days after laser injury. The size of CNV (stained by Isolectin-B4) and fibrosis (stained by anti- type I collagen antibody), subretinal hyperreflective material (a morphological feature seen on nAMD patient) and macrophage infiltration (stained by F4/80 antibody) were quantified. Immunoblot analyses were conducted to examine the phosphorylation of ERK1/2 and SMAD2. *In vitro* study was also performed to confirm the *in vivo* finding. Surgically removed tissues from nAMD patients were used to check the tissue localization of (P)RR. EAU model was induced in B10.BR mice. Clinical and histopathological severities were graded. Retinal-choroidal thickness was examined with spectral domain-optical coherence tomography (SD-OCT). Translocation of NF-κB p65 into the nuclei 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 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 colocalization 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, a morphological feature seen in OCT in nAMD patients (Figure 22). Mechanistically, the blockade of PDGFRβ significantly prevent the recruitment of pericytes to the CNV lesions (Figure 23). In vitro study also supported our in vivo findings that PDGFRβ blockade suppressed PDGF-BB-induced migration of pericytes. To further confirm our *in vivo* finding, we also conducted *in vitro* migration analysis in rat pericytes (TR-rPCT) under the stimulation of PDGF-BB. Notably, the blockade of PDGFRβ suppressed the pericyte migration induced by PDGF-BB in a dose-dependant manner.

(3) In terms of uveitis, IMD-0354 significantly ameliorated pathological changes in EAU mice via inhibition of the NF-κB p65 subunit translocation into EAU retinas. The production of uveitis-related inflammatory cytokines including tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-1α, IL-6, IL-17A, all of which are Th1 and Th17-mediated molecules, shown to increase in human uveitis, was also inhibited *in vitro* when treated with IMD-0354.

【Discussion】The present study, for the first time, provide several finding on the development of novel therapies for nAMD and uveitis. Our group has previously found that (P)RR is associated with the molecular pathogenesis of various disorders such as inflammation and pathological angiogenesis. Most recently, we generated the (P)RR-PshRNA with a single-straned RNAi technique, which has been proved to overcome the drawbacks of canonical double-stranded RNAi agent. Importantly, we have recently confirmed the therapeutic effect of (P)RR-PshRNA in both acute and chronic inflammation model in mice. Herein, 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 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 liver, kidney and spinal cord, it is still unknown whether PDGFRβ signaling contributed to the formation of subretinal fibrosis via peciytes. 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 suppressed 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 may 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.