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学 位 論 文 題 名

Involvement of (pro) renin receptor in the pathogenesis of inflammatory eye diseases
(炎症性眼疾患における(プロ)レニン受容体の病態形成への関与)

【Background and Objectives】 The renin-angiotensin system (RAS) has a key role in the regulation of systemic homeostasis of blood pressure and water balance (circulatory RAS) while several tissues were shown to express the components of RAS (tissue RAS). In tissue RAS, binding of prorenin to (pro)renin receptor [(P)RR] initiates the activation of both RAS and RAS-independent signal transduction in cells. This dual function is referred to as the receptor-associated prorenin system (RAPS) that mediates the molecular pathogenesis of end-organ damage, such as inflammation and angiogenesis, including ocular disorders. Blockades of RAS and/or RAPS have been found to result in beneficial effects on the onset and progression of various ocular diseases some of which were studied extensively by using clinical samples and/or animal models. We hypothesize that RAPS may also play important roles in the molecular mechanism and pathogenesis of various other ocular diseases. Therefore, we focused on RAPS as an alternative target molecular pathway for the treatment of two ocular diseases, extranodal marginal zone lymphoma (EMZL) of the conjunctiva and glaucoma. First, we studied the involvement of RAPS in the molecular pathogenesis of EMZL of the conjunctiva, a tissue that covers the surface of the eyeball and serves as the first defense against pathogens from the outside world. Second, we examined the association of RAPS with the molecular pathogenesis of the trabecular meshwork (TM) of the glaucoma, a major cause of irreversible blindness in the world.

【Methods】 Surgically removed conjunctival EMZL tissues and TM tissues from primary open-angle glaucoma (POAG) and neovascular glaucoma (NVG) patients were used for gene expression, and immunohistochemical (IHC) and immunofluorescence (IF) analyses of RAS components, including (P)RR. Human B-lymphoblast IM-9 cells and human glaucoma TM cells were treated with prorenin or angiotensin II (Ang II), and gene expression levels were analyzed using real-time quantitative PCR (qPCR). IF analysis of EMZL samples and TM from NVG and POAG patient was used to evaluate the *in vivo* expression of protein products the genes with significantly changing expression profiles at prorenin and Ang II stimulations. ELISA was performed to assay the amount of prorenin and Ang II in aqueous humor (AH) from POAG, NVG and cataract patients. TM cells were stimulated with H₂O₂ to induce oxidative stress and mRNA levels of RAS components were assayed by qPCR.

【Results】 Gene expression and IHC analyses revealed the expression of RAS components, including (P)RR and angiotensin II type 1 receptor (AT1R), in EMZL tissues and TM from POAG and NVG patients. IF analyses of EMZL tissues demonstrated that (P)RR and AT1R were detected in cells positive for CD20, a marker for B-cells, where they co-localized with prorenin and angiotensinogen (precursor of Ang II), respectively. Prorenin stimulation of IM-9 cells increased mRNA expression levels of fibroblast growth factor 2 (FGF2), while Ang II treatment increased the expression levels of basigin (BSG), matrix metalloproteinases (MMP)2, 9, and 14, which were abolished by (P)RR and AT1R blockades, respectively. IF analyses of clinical samples showed co-localizations of (P)RR and AT1R with the products of these upregulated genes. The expression of RAS components was observed in TM tissues, and (P)RR and AT1R co-localized with prorenin and angiotensinogen (their respective ligands). Prorenin protein levels in AH from POAG and NVG patients, and Ang II levels in NVG eyes were significantly higher than those of cataract patients. Prorenin stimulation of TM cells increased the expression levels of connexin 43 (CX43) and zona occludens-1 (ZO-1), decreased that of tissue plasminogen activator (PLAT), while Ang II treatment increased the expression level of placental growth factor (PIGF), which were abolished by (P)RR and AT1R blockades, respectively. IF analyses showed co-localizations of (P)RR and AT1R with the protein products of these regulated genes in TM tissues. Oxidative stress led to significant increase in the expression levels prorenin and angiotensinogen in TM cells.

【Discussion】 The present study, for the first time, provides several findings on the association of RAPS in the molecular pathogenesis of two ocular diseases, conjunctival EMZL and glaucoma. Our results indicate that AT1R and (P)RR axes both exist and may be involved with the molecular pathogenesis of EMZL of the conjunctiva and TM of glaucoma. The results of *in vitro* experiments revealed that specific blocking of (P)RR and AT1R diminished the increased expression profiles of the genes involved in the angiogenesis and inflammation (FGF2) and in neovascularization (BSG and MMP-family genes) of EMZL of the conjunctiva. Blocking (P)RR and AT1R also suppressed the increased expression levels of cell junction (CX43 and ZO-1), ECM turnover (PLAT) and angiogenesis (PIGF) related genes, which are thought to contribute to pathologic structural changes in the TM, a major risk factor of glaucoma.

【Conclusion】 (P)RR inhibitors are promising therapeutics to ocular diseases by intervening with the molecular pathway of RAPS in the initial stages and before the clinical conditions become chronic. (Pro)renin receptor blocker (PRRB), one potential (P)RR inhibitors, is a peptide that was designed from tertiary structure of predicted prorenin. Currently PRRB is the only agent to block the prorenin-(P)RR axis of RAPS activation. It has been demonstrated that PRRB potentially suppresses the pathogenesis of various ocular disease models. However, as a peptide, PRRB has limitations like excess amount of peptides required, potential immune response, protease degradation. Future research can be extended to study the inhibitory effects of alternative (P)RR-targeting therapeutic agents on the proliferation and metastasis of conjunctival EMZL by using B lymphocytes and on the pathological changes of cell junction and extracellular matrix in glaucoma mouse models of TM.