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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本研究は、後眼部脈絡膜の発達について遺伝子改変マウスおよびヒト初代培養細胞を用いて詳細な検証実験を積み重ねた結果として、新規分子メカニズムを証明するものである。これまでの研究では、網膜色素上皮細胞(RPE)より分泌される血管内皮増殖因子(VEGF)が脈絡膜形成を担う事が報告されているが、その発現を制御する分子メカニズムはよく分かっていなかった。申請者は、網膜で産生されるレチノイン酸がRPEに作用し、転写因子Sox9を介してVEGFの分泌調節をする事で脈絡膜形成を制御していることを世界で初めて報告した。脈絡膜形成を司る分子機序の解明は加齢黄斑変性や病的近視の病態に関係する重要な課題であり、臨床的にも非常に興味深い成果である。緻密な研究計画に基づき積み重ねられた詳細な実験結果および一連の研究成果は、基礎研究面においても臨床面においても大きな意義を持つものであり、本研究論文は学位に値するものと認める。

論 文 内 容 の 要 旨
Synopsis of Thesis

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論文題名 Title	Neural retina-specific Aldh1a1 controls dorsal choroidal vascular development via Sox9 expression in retinal pigment epithelial cells (神経網膜に特異的なAldh1a1は網膜色素上皮細胞でのSox9の発現を介して、背側の脈絡膜血管発生を制御する)

論文内容の要旨

〔Purpose〕
Vascular endothelial growth factor (VEGF) secreted from retinal pigment epithelium (RPE) cells directs choroidal vascular development. However, the regulation of VEGF secreted from RPE remains incompletely understood. We found that *Aldh1a1* null mutant (*Aldh1a1*$^{-/-}$) mice show choroidal hypoplasia in the dorsal retina. Since Aldh1a1 is responsible for retinoic acid (RA) synthesis, we studied how RAs control VEGF expression in the RPE and investigated the role of Sox9 in developing RPE cells for normal choroidal vascular development.

〔Methods〕
Developmental flat mount immunohistochemistry of RPE and choroids was performed with ZO-1 and Endomucin antibodies to visualize RPE and choroidal vessels, respectively. The VEGF of RPE-choroid complex of embryonic (E) 17.5 wild type (WT) and *Aldh1a1*$^{-/-}$ mice was quantified by ELISA. RA-dependent VEGF expression was examined using primary RPE cells on Transwell membranes. The immunohistochemistry was performed to detect Sox9 in vertical sections of WT and *Aldh1a1*$^{-/-}$ retinas at E12.5 and E14.5. Next, we generated mice with a conditional deletion of *Sox9* by mating either *Tyr*-Cre (*Sox9*^{RPE}-KO) mice or *Pax6*-α-Cre (*Sox9*^{Retina}-KO) mice to delete *Sox9* in the developing RPE and neural retina, respectively. Furthermore, we attempted to rescue the choroidal hypoplasia of *Aldh1a1*$^{-/-}$ mice by restoring Sox9 signaling using Cre-inducible *Sox9*-overexpressing (*Sox9*^{RPE}-OE) mice.

〔Results〕
Choroidal vascular density in the eyes of neonatal and adult *Aldh1a1*$^{-/-}$ mutants was significantly lower than that of WT, indicating reduced vascularization. VEGF levels in the *Aldh1a1*$^{-/-}$ RPE-choroid complex was significantly decreased. RAs significantly enhanced VEGF expression toward the basolateral side of primary RPE cell. The Sox9 intensity of *Aldh1a1*$^{-/-}$ RPE cells was significantly lower than that of WT at E14.5. In *Sox9*^{RPE}-KO mice, we found choroidal hypoplasia in the dorsal region which phenocopied *Aldh1a1*$^{-/-}$ eyes. Conversely, *Sox9*^{Retina}-KO mice showed no hypoplasia of the choroidal vasculature, indicating that Sox9 expression in the neural retina does not affect choroidal vascular development. *Sox9*^{RPE}-OE mice recovered the phenotype of choroidal hypoplasia in the dorsal region.

〔Conclusion〕
These results suggest that RAs produced by Aldh1a1 in the neural retina directs dorsal choroidal vascular development via Sox9 upregulation in the dorsal RPE cells to enhance RPE-derived VEGF secretion.