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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	
<p>本研究は、滲出型加齢黄斑変性の感受性遺伝子であるHTRA1 (high temperature requirement 1) が視細胞死を誘導する機構を明らかにしたものである。加齢や遺伝子変異によって視細胞における発現が亢進するHTRA1をゼブラフィッシュの桿体視細胞特異的に発現させた変異体では、網膜外層における初期加齢黄斑変性様の変化と視細胞死が誘導された。この視細胞死はHTRA1のノックダウンや阻害薬によってレスキューされた。HTRA1は視細胞層のTGF-βを活性化させることで、その下流のAKT-FOXO3の経路を介して抗酸化物質の転写抑制を誘導することが示唆された。これらの知見は、現在の加齢黄斑変性の治療で主流である抗VEGF療法とは全く別の新しい治療法となる可能性がある。よって本研究は学位に値するものと認める。</p>	

論 文 内 容 の 要 旨
Synopsis of Thesis

氏 名 Name	大浦 嘉仁
論文題名 Title	HTRA1 causes photoreceptor cell death in zebrafish disease models (滲出型加齢黄斑変性感受性遺伝子HTRA1は視細胞死を誘導する)
<p>[目的(Purpose)]</p> <p>Age-related macular degeneration (AMD) is an important cause of blindness. It is characterized by a retinal pigment epithelium (RPE) disorder that leads to death of photoreceptor cells (PRCs). AMD has a strong genetic association with high temperature requirement A 1 (HTRA1). The relationship between HTRA1 and the AMD phenotype is unknown. The purpose of this study is to clarify the physiological expression and function of HTRA1 in retina and to evaluate an influence of HTRA1 on rod photoreceptor cells using transgenic zebrafish specifically overexpressing human HTRA1 in rod photoreceptor cells.</p> <p>[方法ならびに成績(Methods/Results)]</p> <p>We examined the expression assay for localization and change with aging in protein and mRNA levels in zebrafish and mouse by immunostaining, immunoblotting, quantitative PCR and we investigated how SNP mutation of human HTRA1 promotor has an effect on expression of HTRA1 in vivo by Met-luc reporter assay using two promotors with different length in ARPE-19 and Y79. We showed that the expression of <i>HTRA1</i> in PRCs, as well as in RPE, is increased by aging and the disease-associated <i>HTRA1</i> mutation. Next, we generated transgenic zebrafish specifically overexpressing human HTRA1 in rod photoreceptor cells (<i>Tg(rho:hsa.HTRA1)</i>) and evaluated the phenotype by transmission electron microscope and survivability assessment of rod PRCs by counting number of rod photoreceptor cells and TUNEL assay. <i>Tg(rho:hsa.HTRA1)</i> showed morphological changes of the RPE and PRC layer similar to those of early AMD in transmission electron microscope images. We identified the reduction of rod PRCs in <i>Tg(rho:hsa.HTRA1)</i> due to apoptosis by TUNEL assay and quantitative polymerase chain reaction (PCR) of apoptosis-associated caspases. We also confirmed the increase of <i>htrala</i> expression in a retinitis pigmentosa zebrafish model. In both fish lines, PRC death was rescued by the suppression of <i>htrala</i> by the HTRA1 inhibitor (6-boroV). Moreover, we analyzed how overexpression of HTRA1 effected on TGF-β signaling with checking mRNA and protein expression of its downstream gene by quantitative PCR, immunohistochemistry and immunoblotting for zebrafish and found that AKT-FOXO3 signaling was activated via TGF-β activation by increased HTRA1, resulting in PRC death.</p> <p>[総括(Conclusion)]</p> <p>Our findings suggest that HTRA1 overexpressed in PRC induces PRC death and morphological changes in retina like early AMD and up-regulates TGF-β-AKT-FOXO3 probably contributed to PRC death. HTRA1 is a potentially effective target for neuroprotective therapy of early AMD and other degenerative diseases of PRCs.</p>	