Effects of Mitochondrial Ferritin (FTMT) Expression by Retinal Pigment Epithelial Cells on Features of Angiogenesis.

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別紙様式３（課程博士・論文博士共用）

論　文　内　容　要　旨

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学位論文題目	Effects of Mitochondrial Ferritin (FTMT) Expression by Retinal Pigment Epithelial Cells on Features of Angiogenesis (網膜色素上皮細胞によるミトコンドリアフェリチンの発現が血管新生に及ぼす影響)		

[Research Background]

Pathological ocular angiogenesis/neovascularization, particularly in the retina and choroid, should be carefully controlled as it may lead to significant visual impairment. Age-related macular degeneration (AMD), a leading cause of vision loss, can result from pathological angiogenesis. As a mutation in the mitochondrial ferritin (FTMT) gene has been associated with AMD, its possible role in modulating angiogenic factors and angiogenesis was investigated. FTMT is an iron-sequestering protein primarily expressed in metabolically active cells and tissues with high oxygen demand, including retina. In a previous study we revealed that age-related increases of FTMT in murine retina. A number of studies have demonstrated that FTMT may have multiple properties, such as protective roles against oxidative stress and hypoxia in neuronal cells. Although expression of FTMT is usually very low to undetectable in most cell types, it is expressed at detectable levels in RPE cells.

[Aim of this study]

To examine the consequences of manipulating FTMT expression in RPE cells on expression of angiogenic factors including vascular endothelial growth factor (VEGF), and effects on angiogenesis.

[Materials and methods]

In this study, we utilized the human retinal pigment epithelial (RPE) cell line ARPE-19 to investigate interactions of FTMT and angiogenesis. First, we compared differentiated and undifferentiated ARPE-19 cells to extend the relevance of this model for FTMT expression. To be able to study the effect of FTMT on RPE cell phenotype, we produced stably transfected FTMT overexpressing cells. The effects of proinflammatory cytokines (TNF-α, IL-1β and IFN-γ), FTMT knockdown, and transient and stable overexpression of FTMT were investigated on expression of pro-angiogenic VEGF and anti-angiogenic pigment-derived epithelial factor (PEDF).

（備考）１．論文内容要旨は、研究の目的・方法・結果・考察・結論の順に記載し、２千字程度でタイプ等を用いて印字すること。
２．※印の欄には記入しないこと。

We were also able to demonstrate in vitro tube formation assay that an altered pattern of secretion of angiogenic factors from FTMT overexpressing cells. We employed the hCMEC/D3 human cerebrovascular endothelial cell line as the indicator cell type in the in vitro angiogenesis tube formation assay and cell proliferation assay. Experiments were carried out with media from FTMT overexpressing and vector transfected cells and were used undiluted *(100)* or diluted *(50:50)* with original media.

[Result and discussion]

We demonstrated that proinflammatory cytokines TNF-α, IL-1β and IFN-γ induced FTMT and VEGF transcription in ARPE-19 cells and VEGF secretion. As increased VEGF promotes angiogenesis in a number of retinal diseases, such as AMD and diabetic retinopathy, it has made VEGF a highly significant therapeutic target. Since both FTMT and VEGF mRNA, and VEGF secretion were significantly upregulated by TNF-α in ARPE-19 cells, we next investigated the association between them by using siRNA to inhibit FTMT gene expression and FTMT overexpression in ARPE-19 cells. FTMT gene silencing increased VEGF secretion compared to the control group. In contrast, the overexpression of FTMT served to reduce VEGF mRNA and protein expression in both TNF-α treated and untreated cells. Our results suggested that FTMT has an inhibitory effect on VEGF secretion in ARPE-19 cells. However, as the overexpression of FTMT did not abolish TNF-α induced increase in VEGF mRNA and protein secretion, this would suggest that the link between FTMT and VEGF could be indirect through multiple signaling mechanisms.

FTMT overexpression increased levels of mRNA for the differentiation marker retinal pigment epithelial-specific 65kDa protein RPE65. This could be related to the antioxidant properties of FTMT, but further studies are needed. Key findings were the inhibition of VEGF expression and increases of PEDF expression in RPE cells overexpressing FTMT. The effects of FTMT were evident in an in vitro angiogenesis assay, that demonstrated that conditioned media from FTMT overexpressing cells significantly inhibited most of the in vitro tube features of angiogenesis in brain endothelial cells. However, based on the current state of knowledge of the multiple features of FTMT, one can hypothesize that its potent antioxidant properties could be modulate gene expression of angiogenic and inflammatory factors in RPE cells, even under normal conditions.

[Summary]

From these findings, it can be concluded that FTMT has an inhibitory effect on VEGF expression and secretion in ARPE-19 cells; alters the phenotypes of overexpressing cells; and alters the secreted angiogenic factors from overexpressing cells, resulting in inhibition of angiogenesis.

別紙様式９（課程博士・論文博士共用）

博士論文審査の結果の要旨

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（博士論文審査の結果の要旨）

本論文では、加齢黄斑変性にmitochondria Ferritin (FTMT)の遺伝子異常を持つ患者があることや老化によって網膜でのFTMT発現量が増加することから、FTMTが網膜の血管新生に関係するかとの疑問を解明するために研究をおこなった。ARPE-19 細胞, hCMEC/D3 細胞を用いて、VEGF, PEDF産生への影響、tube formationなどの手法を用いて検討を行い、以下の点を明らかにした

1） FTMT mRNAはARPE-19 細胞の分化の影響を受けなかった。
2） Pro-inflammatory cytokines (TNF-α, IL-1β, IFN-γ)の刺激により、ARPE-19細胞によるFTMT mRNA, VEGF mRNA, VEGF proteinの産生が亢進した。
3） ARPE-19細胞ではFTMTの抑制により、VEGF mRNA, VEGF proteinの産生が亢進した。
4） FTMTを過剰発現させたARPE-19細胞では、VEGF mRNA, VEGF proteinともに産生が抑制された。
5） Differentiated ARPE-19細胞ではFTMT過剰発現によりPEDF mRNAが増加したが、undifferentiated ARPE-19細胞では変化がなかった
6） FTMT過剰発現させたARPE-19の培養液を用いたconditioned mediaによりhCMEC/D3細胞によるtube formationが抑制された。
7） 結論として、FTMT増加により血管新生が抑制された。おそらく血管新生を促進する因子であるVEGF産生抑制と血管新生を抑制する因子であるPEDF産生亢進を介した機序が考えられた。

本論文は、網膜色素上皮における FTMT の血管新生への影響について新たな知見を与えたものであり、また最終試験として論文内容に関連した試問を実施したところ合格と判断されたので、博士（医学）の学位論文に値するものと認められた。

（　令和３年１月２７日）