論 文 内 容 の 要 旨
Synopsis of Thesis

氏 名 Name	原田 佳奈
論文題名 Title	Repulsive guidance molecule A suppresses angiogenesis (RGMaによる血管新生抑制)

〔Purpose〕

Angiogenesis is the process of new blood vessel formation from pre-existing vessels, Angiogenesis can be promoted by various growth factors, and vascular endothelial growth factor (VEGF) has been shown to play a dominant role. The repulsive guidance molecule A (RGMa) is a membrane-associated glycoprotein originally identified an axon repellent in the visual system. Neogenin serves as a receptor for RGM. RGMa binds to neogenin and mediates its repulsive activity toward retinal axons allowing it to play roles in both the developing and adult central nervous systems. These findings prompted us to hypothesize that RGMa may act as a functional cue in angiogenesis.

〔Methods/Results〕

In this study, we show that RGMa suppresses pathological angiogenesis. *In vitro*, to characterize the role of RGMa in angiogenesis, we examined its effects on endothelial tube formation and migration. The treatment of HUAEC with VEGF increased tube formation, which was inhibited by RGMa. RGMa also significantly inhibited the ability of endothelial cells to form tube-like vessels in Matrigel. We then performed loss-of-function experiments to assess whether neogenin is necessary for the inhibitory effect of RGMa on tube formation. Neogenin expression was effectively and specifically silenced at the mRNA and protein levels in neogenin siRNA-transfected cells. Knockdown of neogenin in HUAEC attenuated the suppressive effect of RGMa on tube formation, even though neogenin knockdown alone increased tube formation. As these effects of RGMa are dependent on neogenin, we examined the subcellular localization of neogenin in HUAEC. Although the neogenin signal was localized in nuclei and the cytoplasm in unstimulated cells, treatment with RGMa resulted in intense localization of the neogenin signal in the nuclei and disappearance of the signal in the cytoplasm. Consistent with these findings, RGMa attenuated VEGF-induced phosphorylation of FAK at tyrosine-397. Finally, to investigate the anti-angiogenic activity of RGMa *in vivo*, we performed Matrigel plug assays in ICR female mice, a typical model for analyzing angiogenesis. Matrigel was mixed with VEGF-A in the presence or absence of RGMa, aliquots of the mixture were subcutaneously injected into the flanks of the mice, and Matrigel plugs were collected 7 days after injection. Notably, the inclusion of RGMa in Matrigel attenuated VEGF-induced capillary blood vessel formation in the plug. The relative level angiogenesis was also evaluated by hemoglobin content in the Matrigel plug. Inclusion of RGMa in the VEGF-treated Matrigel reduced the hemoglobin level compared to the VEGF-treated control. Collectively, these results demonstrate that RGMa suppresses VEGF-induced angiogenesis *in vivo*.

〔Conclusion〕

RGMa is involved in angiogenesis and can suppress new blood vessel formation both *in vitro* and *in vivo*. Treatment of HUAEC with recombinant RGMa inhibited vascular endothelial growth factor VEGF-induced tubular formation and migration. Knockdown of neogenin in HUAEC abolished the inhibitory effect of RGMa on tubular formation. In conclusion, RGMa inhibits pathological angiogenesis and suggest that its manipulation would be an alternative therapeutic strategy for pro-angiogenic conditions.

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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

申請者は、Neogeninを受容体とするNetrin-4の血管新生抑制作用に着目し、同様にNeogeninを受容体とする神経再生阻害因子である Repulsive guidance molecue (RGMa)の血管系に対する作用を解明することを目的に研究を行った。学位論文において申請者は、神経再生阻害因子であるRGMaが、血管新生を抑制することを明らかにしている。具体的には、血管内皮細胞HUAECを用いたMatrigel tube formation assayにおいて、RGMaがtube formationを抑制すること、細胞遊走を抑制することが示された。この効果は、Neogenin siRNAを導入した細胞ではブロックされたため、RGMaがNeogeninを介して血管新生を抑制することが示唆された。本研究は、神経軸索の再生阻害を司るRGMa-Neogeninシグナルが、血管内皮細胞においては、血管新生を抑制することが明らかとなったという点で、軸索再生阻害タンパク質の新たな機能を提唱するものであり、博士(医学)の学位授与に値すると考えられる。