Investigating the Role of Repulsive Guidance Molecule C on Central Nervous System Angiogenesis

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Introduction

- Angiogenesis is the formation of new blood vessel sprouts from existing vasculature
- Molecules and mechanisms that guide central nervous system (CNS) angiogenesis is of interest due to its therapeutic values[1]
  - Decreasing/inhibiting angiogenesis can be therapeutic in diseases: cancer, diseases of the eye, wound healing[1]
- Repulsive guidance molecule C (RGMc) has been shown to affect retinal angiogenesis[2]
  - RGMc is produced by the liver and muscle[2]
  - RGMc KO mice experience abnormal vasculogenesis and angiogenesis in retina[3]
  - Differences between liver and muscle derived RGMc on CNS angiogenesis has not been characterized
- Preliminary results form our lab shows that RGMc produced by the liver or muscle have opposing effects on retinal angiogenesis

Hypothesis

Liver and muscle derived RGMc have opposing effects on retinal angiogenesis. We speculate that this difference could be due to post-translational modifications of the protein.

Objectives

Qualitative and Quantitative analysis of retinal vessel plexus in liver RGMc ($RGMc^{\Delta Alb\text{-}Cre}$) and muscle RGMc ($RGMc^{\Delta Acta\text{-}Cre}$) cre loxp mouse model

1. Ex-vivo imaging using two-photon excitation microscopy to assess P11 vasculature

2. In-vitro experiments using purified RGMc proteins from liver an muscle cell lines for tube formation assay to support ex-vivo results

3. Assessment of post-translational modifications of RGMc that could lead to differences in retinal angiogenesis regulation
   - Thrombin is a possible post-translational factor that can modulate RGMc (RGMc contains thrombin cleavage site)

Materials & Methods

1. Ex-vivo two-photon imaging

Transgenic KO mice with their respective controls (C57BL/6J) at P11 anaesthetized and perfused

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Whole mount and immunostaining of retinal vasculature

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3D configuration of retinal vasculature was acquired by two-photon imaging

2. Post-translational modifications of RGMc

Transfection and purification of RGMc from HEK cells

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Reaction mixture of RGMc + Thrombin (hormone secreted by liver)

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Western blot analysis to determine if RGMc is cleaved/modified by thrombin

3. In-vitro experimentation (tube formation assay)

RGMc plasmid transfection and into AML12 and C2C12 cell lines and protein purification

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HUVEC cells used for tube formation assay to generate vascular network

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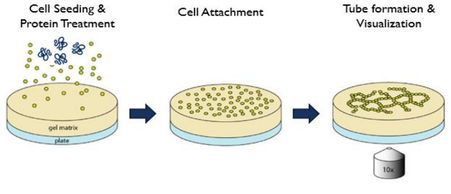
HUVEC cell treatment with RGMc from liver and muscle RGMc as well as RGMc cleaved with thrombin to determine effects on angiogenesis



Results

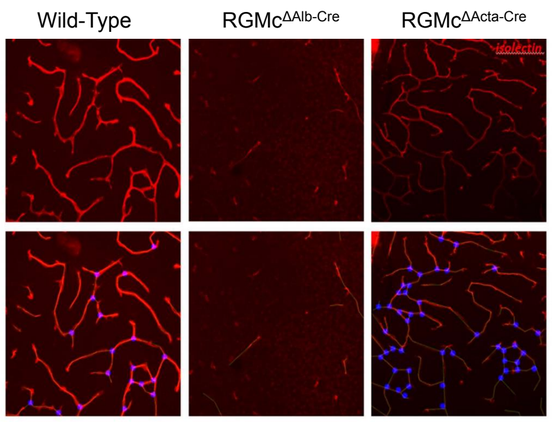


Figure 1. Representative two-photon images of the intermediate vessel plexus stained with isolectin in wildtype, $RGMc^{\Delta Alb\text{-}Cre}$, and $RGMc^{\Delta Acta\text{-}Cre}$ mouse retina. Average number of branch points (blue dots) are significantly higher in $RGMc^{\Delta Acta\text{-}Cre}$ (51.67) compared to both wildtype (16.57) and $RGMc^{\Delta Alb\text{-}Cre}$(1.17). Total length of blood vessels determined to be 1823.43µm, 478.83µm, and 3053.67µm for wildtype, $RGMc^{\Delta Alb\text{-}Cre}$, and $RGMc^{\Delta Acta\text{-}Cre}$ respectively. (P<0.001)

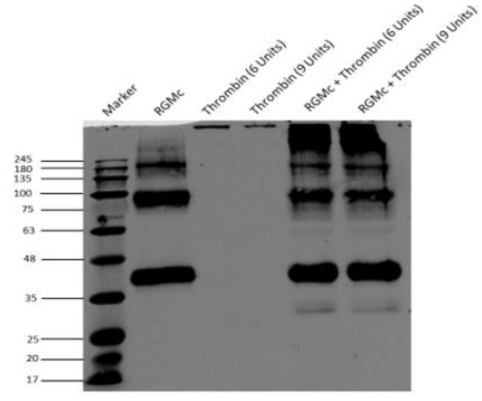


Figure 2. Western Blot Analysis of RGMc Treated Thrombin (12% Gel). Bands at 30 kDa detected in Lanes 5 & 6 with no band indicating active thrombin cleavage site on RGMc. Higher frequency of bands located at top of the gel in Lanes 5 & 6 suggesting that thrombin leads to increased RGMc polymerization.

Discussion & Conclusion

- Liver and muscle derived RGMc have opposing effects on angiogenesis, one acting as a pro-angiogenic factor and the other as an anti-angiogenic factor respectively, affecting retinal vasculature development/formation
- Post-translational modifications of RGMc could be the cause of the difference effects that liver vs muscle derived RGMc has on retinal angiogenesis
  - Thrombin can cleave RGMc and also aid its polymerization → may lead to differences in angiogenic effects seen in retinal vasculature
- In-vitro tube formation assay currently requires modifications in order to obtain results and further support hypothesis

Future directions

- Expansion of ex-vivo experiments in mice at different ages
- Determine if angiogenesis in other areas of the CNS are affected in similar ways to the retina
- Identify underlying mechanisms and possible receptors of RGMc (e.g. NEO1 & BMP) that can better explain this phenomenon

References

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