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

Michal Syonov[1], Xinyi He[3], and Philippe P. Monnier[1,2]

[1]Department of Physiology, *University of Toronto,* [2]Division of Genetics and Development, Krembil Discovery Tower, [3]Institute of Biomedical Engineering, *University of Toronto*

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] Inhibiting angiogenesis is 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 tissue[2], RGMc binds to receptors Neogenin (NEO1) & Bone Morphogenic Protein (BMP)[2], RGMc KO mice experience abnormal vasculogenesis and angiogenesis in the retina[3]
- Differences between liver and muscle derived RGMc on CNS angiogenesis have not been characterized. Our lab shows that RGMc produced by the liver or muscle have distinct effects on angiogenesis

HYPOTHESIS

Liver- and muscle-derived RGMc have opposing effects on angiogenesis, acting as pro-angiogenic and anti-angiogenic factors, respectively. This difference could be due to modifications of the protein and resultant differences in receptor interaction.

OBJECTIVES

1. Ex-vivo imaging using two-photon excitation microscopy to assess P6-P11 retinal vasculature in liver ($RGMc^{\Delta AlbCre}$) and muscle ($RGMc^{\Delta ActaCre}$) Cre-lox mouse model
2. In-vitro tube formation assay using purified RGMc proteins from liver and muscle cell lines to support ex-vivo results
3. Assessment of modifications of RGMc and possible differences in interactions with its receptors NEO1 & BMP

MATERIALS & METHODS

1. Ex-vivo confocal imaging of retina
   - $RGMc^{\Delta AlbCre}$ and $RGMc^{\Delta ActaCre}$ mice with respective littermate controls were anaesthetized and perfused for whole mount immunostaining of retinal vasculature
2. In-vitro tube formation assay
   - Transfection & protein purification of RGMc plasmid using AML12 & C2C12 cell lines
   - HUVEC cell seeded on Matrigel matrix to generate vascular network and protein treatment incubated with cells for 12 hours
3. Assess differences between liver & muscle derived RGMc (western blot & sequencing)

RESULTS

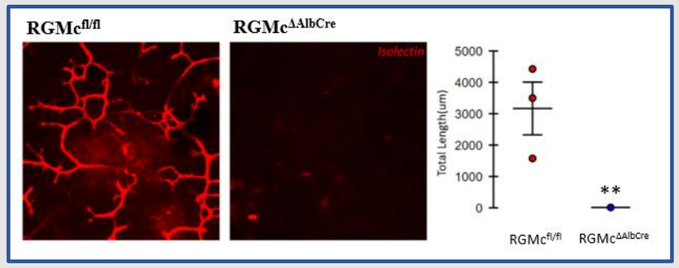

Fig 1. Confocal Images of Retinal Intermediate Vessel Plexus in P11 $RGMc^{fl/fl}$ and $RGMc^{\Delta AlbCre}$ Mice. Within the area of 425.1µm x 425.1µm in the temporal quadrant of the intermediate vessel plexus in P11 $RGMc^{fl/fl}$ and $RGMc^{\Delta AlbCre}$ mice retina, the average total length is 1440µm and 377µm, respectively ($p<0.01$).

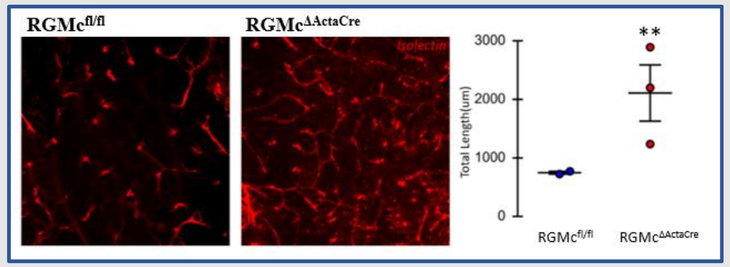

Fig 2. Confocal Images of Retinal Intermediate Vessel Plexus in P11 $RGMc^{fl/fl}$ and $RGMc^{\Delta ActaCre}$ Mice. Within the area of 425.1µm x 425.1µm in the temporal quadrant of the intermediate vessel plexus in P11 $RGMc^{fl/fl}$ and $RGMc^{\Delta ActaCre}$ mice retina, the average total length is 748µm and 2108µm, respectively ($p<0.01$).

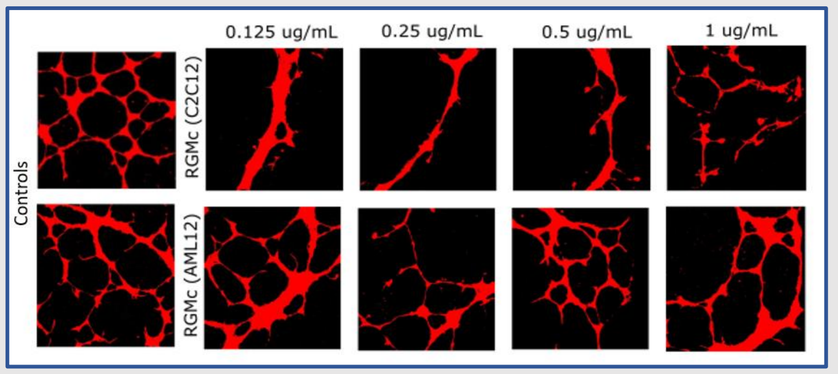

Fig 3. HUVEC Cell Tube Formation Assay Using Muscle & Liver Derived RGMc Protein Treatments. Muscle derived RGMc treated cells showed a decrease in angiogenesis (vessel length & no. of branch points) while liver derived RGMc treated cells seem to have no effect on angiogenic activity. This indicates that muscle derived RGMc acts as an anti-angiogenic molecule while liver derived RGMc may act to maintain angiogenesis ($p<0.05$).

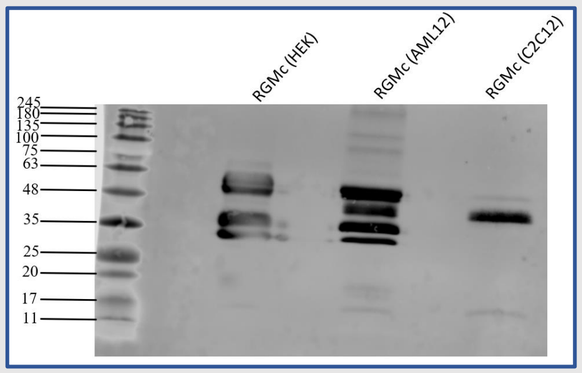

Fig 4. Western Blot Analysis Comparing Liver and Muscle Derived RGMc (12% Gel). Lanes 2 & 3 show both 50 kDa & 40 kDa soluble forms of the protein and the membrane bound 50 kDa & the 35 kDa/20kDa isoform formed by cleavage of the GDPH autocatalytic site. Lane 4 only presents the soluble forms and 50 kDa membrane bound form of RGMc without the 35kDa/20kDa fragments, signifying that muscle derived RGMc cannot be cleaved at its autocatalytic cleavage site.

CONCLUSIONS & FUTURE DIRECTIONS

- Liver & muscle derived RGMc have different effects on angiogenesis shown both in ex-vivo and in-vitro experiments
- Identified a difference between liver and muscle RGMc via western blot analysis, where muscle derived RGMc cannot be cleaved at its GDPH autocatalytic site, which can possibly explain the difference between these proteins (sequencing required to support these results)
- Expansion of ex-vivo experiments in the rest of the CNS
- Generate an RGMc construct with a point mutant at the GDPH site and using it in the tube formation assay
- Identify underlying mechanisms and possible receptor interactions with RGMc (e.g., NEO1 & BMP) that can better explain this phenomenon

REFERENCES

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