Determination of the effect of microglia-derived olfactomedin-like 3 on primary mouse brain endothelial cell angiogenesis

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Introduction

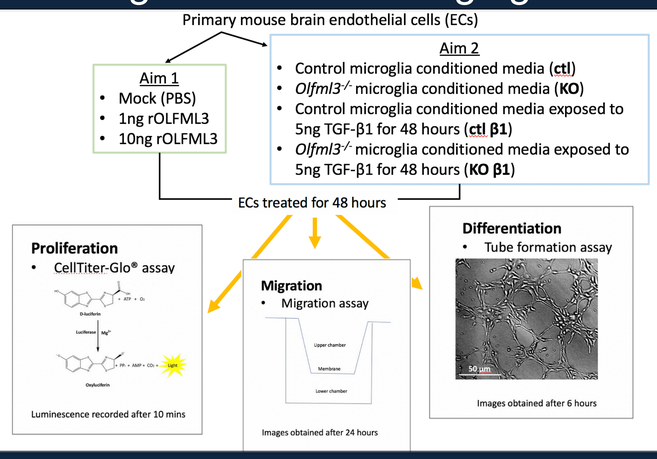
Glioblastoma multiforme (GBM) is a uniformly fatal brain cancer in humans and canines. Within GBM, microglia comprise up to 50% of the tumor mass. Under the influence of tumor-derived transforming growth factor-beta (TGF- β1), glioma associated microglia promote new blood vessel formation. It is well established that olfactomedin-like 3 (OLFML-3), a secreted glycoprotein, promotes non-CNS angiogenesis. Of the cell types within GBM that express OLFML-3, only microglia increase OLFML-3 expression in response to TGF- β1. Despite this evidence, the role of microglia-derived OLFML-3 within glioma remains unknown. We hypothesize that TGF- β1 induced microglia-derived OLFML3 expression in microglia cells promotes primary mouse brain endothelial cell (EC) angiogenesis.

Aims

Aim 1A: Determine whether rOLFML3 is capable of increasing primary mouse brain endothelial cell angiogenesis.

Aim 1B: Determine whether conditioned microglial media, utilizing *Olfml3*$^{-/-}$ and isogenic control microglia, differentially increases primary mouse brain endothelial cell angiogenesis.

Testing the events of angiogenesis



rOLFML3 increased EC proliferation and migration

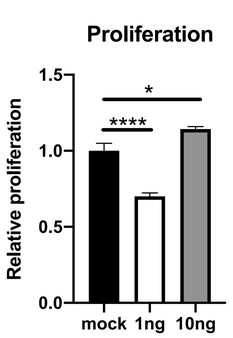
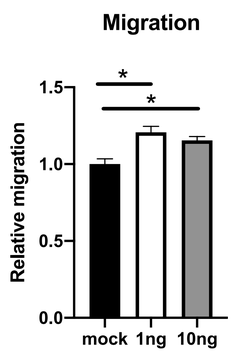


Figure 1: Low dose rOLFML3 decreased while high dose rOLFML3 increased primary mouse brain EC proliferation. Primary mouse brain EC proliferation decreased following 48-hour exposure to 1ng rOLFML3 (29.9%; p<0.0001) but increased following exposure to 10ng rOLFML3 (14.3%; p<0.05).

Figure 2: rOLFML3 increased migration in primary mouse brain ECs. EC migration increased following 48-hour exposure to 1ng (20.7%; p<0.05) and 10ng (15.5%; p<0.05) rOLFML3.

(Data is represented at group mean normalized to mock treated group +/- SEM)



rOLFML3 variably affected EC differentiation

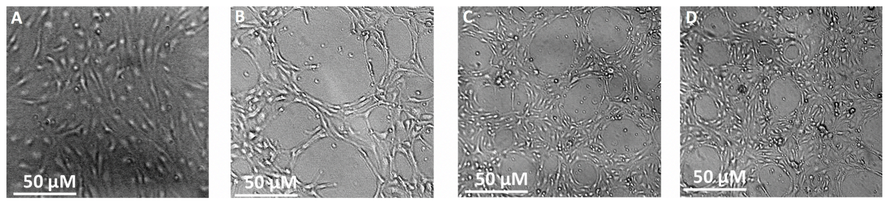


Figure 3: rOLFML3 alters primary mouse brain EC differentiation. A) representative image of ECs growing in culture in a monolayer B) ECs in the presence of Matrigel, an extracellular matrix material. These ECs formed long, branching tubes that are 1-4 cell layers thick and create geometric shaped loops. C) ECs in the presence of Matrigel and 1ng rOLMFL3. These ECs formed highly cellular tubes (4+ cell layers) that were short, exhibited frequent branching and formed small, circular loops. D) ECs in the presence of Matrigel and 10ng rOLFML3. These ECs were highly cellular, short and exhibited frequent branching. The loops were the smallest and most circular compared to the other conditions.

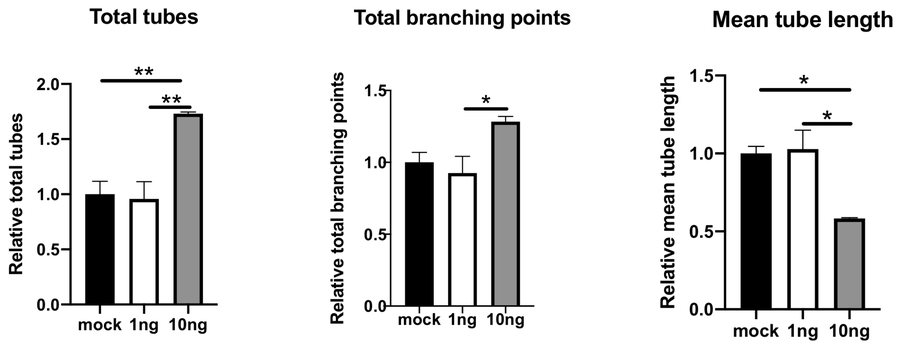


Figure 4: High dose rOLFML3 increased total tubes but decreased average tube length. High dose rOLFML3 increased total tubes (73%; p<0.01) but decreased mean tube length (41.7%; p<0.05) relative to the control group.

Conditioned media (CM) from isogenic control (ctl) and *Olfml3*$^{-/-}$ (KO) microglia variably affected EC proliferation and migration

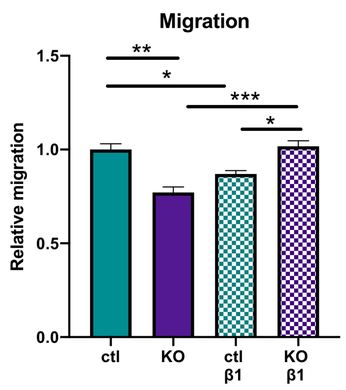


Figure 5: CM from KO and TGF-β1 treated control microglia decreased primary mouse brain EC migration. Migration of primary mouse brain ECs decreased (22.9%; p<0.01) following 48-hour exposure to *Olfml3*$^{-/-}$ microglia CM. EC migration also decreased (13%; p<0.05) following 48-hour exposure to control microglia TGF-β1 treated CM.

(Data is represented as group mean normalized to control CM group +/- SEM)

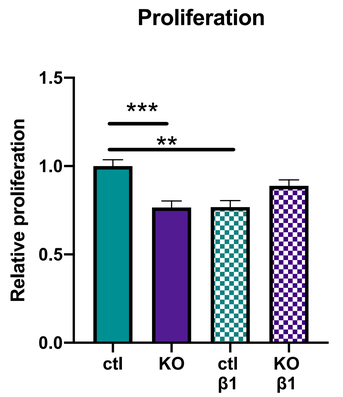


Figure 6: CM from KO microglia and TGF-β1 treated control microglia CM decreased primary mouse brain EC proliferation. EC proliferation decreased (23.4%; p<0.001) following 48-hour exposure to *Olfml3*$^{-/-}$ microglia CM relative to exposure to control microglia CM. EC proliferation decreased (23.2%; p<0.01) following 48- hour exposure to TGF-β1 treated control microglia CM relative to control microglia CM.

TGF-B1 pre-treatment decreased EC differentiation

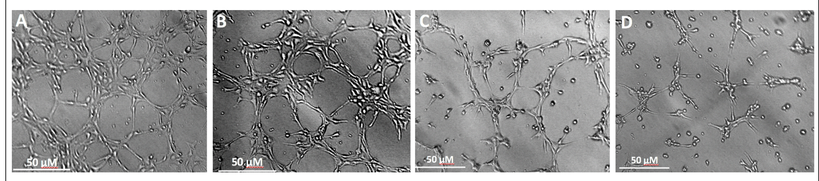


Figure 7: ECs exposed to TGF-β1 treated microglia CM decreased tube formation. A) A representative image of ECs exposed to isogenic control microglia CM. ECs formed long, branching tubes with geometric shaped loops. B) ECs exposed to *Olfml3*$^{-/-}$ microglia CM form similar tube-like structures as ECs exposed to control microglia CM. C) ECs exposed to TGF-β1 treated control microglia CM had reduced tube formation relative to control microglia CM. D) ECs exposed to TGF-β1 treated *Olfml3*$^{-/-}$ microglia CM had reduced tube formation relative to control microglia CM.

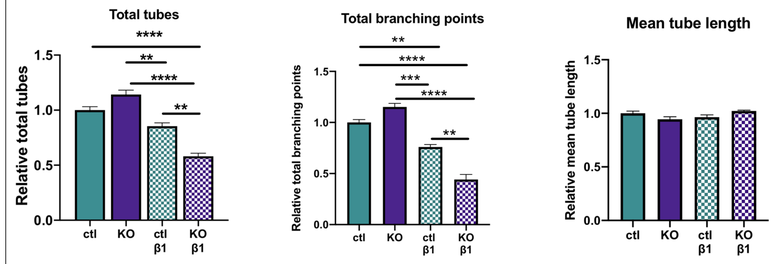


Figure 8: TGF-β1 treated microglia CM reduced primary mouse brain EC tube formation. No parameters of differentiation were altered between ECs exposed to isogenic control microglia CM and ECs exposed to *Olfml3*$^{-/-}$ microglia CM. TGF-β1 treated control microglia CM reduced EC total branching points (24%; p<0.01). TGF-β1 treated *Olfml3*$^{-/-}$ microglia CM reduced EC total branching points by (61.7%; p<0.0001) and reduced total tubes by (49%; p<0.0001) relative to ECs cultured with control microglia CM.

(Data is represented as group mean normalized to control CM group +/- SEM)

Conclusions

- The effect of OLFML3 on all phases of primary mouse brain EC angiogenesis is complex and variable.
- OLFML3 may have a dose dependent effect on angiogenic parameters
- Therefore, it is possible that OLFML3 has a homeostatic function in EC angiogenesis.
- OLFML3 may not affect all parameters of angiogenesis equally. It seems the differentiation phase of angiogenesis is not affected by a loss of OLFML3 secreted by microglia, however there may be other angiogenic signaling molecules that are upregulated and secreted by *Olfml3*$^{-/-}$ microglia that compensate for the loss of OLFML3.
- Overall, OLFML3, whether exogenous or endogenous, affected primary mouse brain endothelial cell angiogenic parameters when compared to control EC angiogenesis assays. As a result, OLFML3 should be investigated further as a novel anti-angiogenic therapeutic target.

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