Final Report

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Title of Project: The ability of progranulin to acutely decrease blood brain barrier permeability, attenuate brain injury and reduce functional impairment following experimental stroke.

Summary:
The goal of this project was be to evaluate the therapeutic attributes of progranulin for the acute treatment of ischaemic stroke by exploring the influence of treatment with progranulin on blood vessel leakiness and the impact of stroke. The first aim was to evaluate the ability of treatment with progranulin (recombinant protein, injected intravenously) to reduce ischemic brain injury in response to ischemic stroke. Stroke was induced in C57Bl/6J mice using the technique of intraluminal middle cerebral artery occlusion (MCAO) and mice were treated with either Vehicle or progranulin (100, 200ng) at reperfusion (0hrs). As shown in Figure 1, we did not observe a protective effect of progranulin, as infarct volume, oedema and behavioural function. Deficits were similar in all three groups (**Fig.1**).

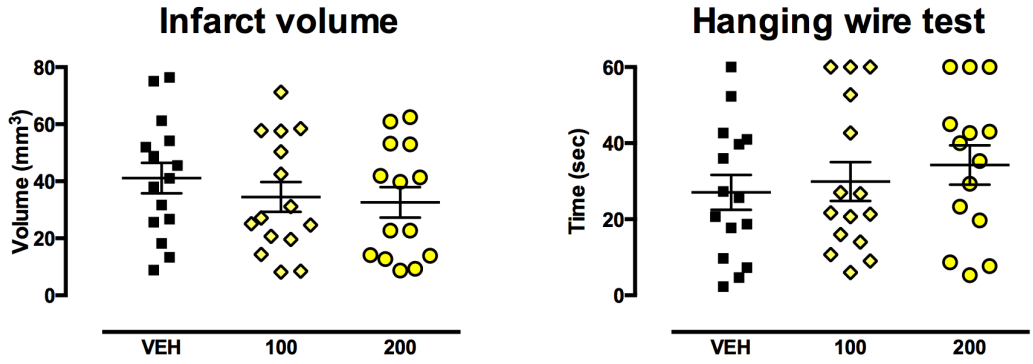

Fig. 1. Treatment with progranulin (100, 200 ng) upon induction of reperfusion failed to exert neuroprotection against ischaemic stroke.

Given this negative finding we decided to redirect the focus of the study on the effects of progranulin on blood brain barrier (BBB) integrity. In collaboration with Robert Medcalf, we characterized the ability of progranulin to stabilize vascular integrity using an in vitro BBB model[1]. We demonstrated that progranulin can rapidly promote BBB integrity in response to combined permeability insults including oxygen glucose deprivation (**Fig. 2A)** and combined tissue plasminogen activator (**Fig. 2B**). These and other complementary findings support the therapeutic benefit of using progranulin to increase BBB integrity in response to injury. By adding progranulin selectively to the luminal (endothelial) or abluminal (astrocyte) side of the transwell system, we demonstrated that both endothelial cells and astrocytes contribute to the vascular stabilizing effects (**Fig. 2A**). We have shown that progranulin prevents changes in endothelial cell morphology that occur in response to permeability insults (data not

shown). In addition, we have confirmed that the recently described progranulin binding partner sortilin does not mediate these effects (data not shown).

Collectively, these findings do not support the usefulness of progranulin as a treatment for acute stroke. However, they do support beneficial effects of progranulin on blood brain barrier integrity. While progranulin may not be able to offset the massive degree of BBB disruption that occurs in stroke, it may be valuatble in diseases in which the degree of BBB disruption is more modest and sustained (e.g. multiple sclerosis). These findings are currently being prepared for publication and will be submitted this year.

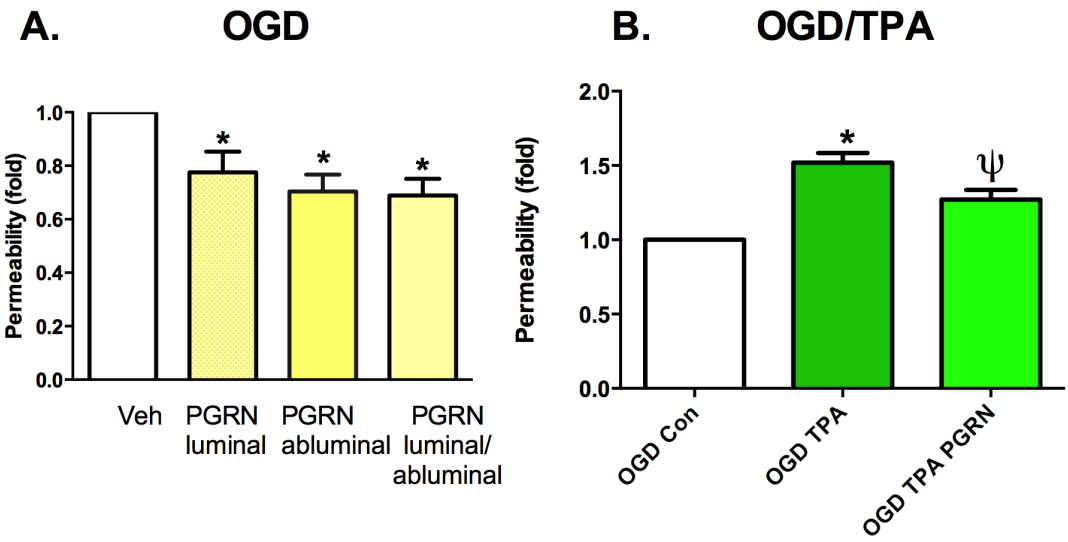

Fig. 2. Investigating the BBB stabilizing effects of progranulin using an in vitro system. A. Progranulin (400 ng) attenuates BBB permeability in response to oxygen glucose deprivation. Interestingly, progranulin stabilizes the BBB when administered to either the luminal (endothelial) or abluminal (astrocyte) side of the transwell, suggesting that both cell types contribute to vascular stability (n=8, *P<0.05, ANOVA). **B.** Progranulin attenuates BBB permeability in response to combined oxygen glucose deprivation and tissue plasminogen activator(tPA, n=7, *P<0.05 versus control, Ψ versus OGD tPA, ANOVA).

References

1. Niego B, Freeman R, Puschmann TB, Turnley AM, Medcalf RL. t-PA-specific modulation of a human blood-brain barrier model involves plasmin-mediated activation of the Rho kinase pathway in astrocytes. *Blood*. 2012;119:4752–4761.