Glypisomes: A Novel Construct for Enhancing Angiogenic Activity of Delivered Growth Factors

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Statement of Purpose: Ischemia is a common consequence of peripheral vascular disease, which affects more than 27 million patients in the United States. Currently, surgical therapies exist for treatment of ischemic disease, but these treatments are prone to failure in the long term. Regenerative therapies that stimulate the growth of new vasculature have great potential for treating peripheral and myocardial ischemia. Growth factor based therapies that induce neovascularization have been successful in animal models, but have limited success in clinical trials. Here, we have developed a new method for enhancing the activity of growth factors in growth factor resistant disease states such as diabetes and hyperlipidemia. Our novel method delivers the growth factor co-receptor glypican-1 embedded in a liposomal carrier to create a glypican-1 proteoliposome (a "glypisome").

Methods: Liposomes were made by resuspending a dried lipid film and extruding it through a 400 nm polycarbonate filter. Purified glypican-1 (48.8 µg/ml) was added to the liposome solution to create the glypisomes. We tested the ability of glypisomes to enhance FGF-2 and VEGF activity in in-vitro models of endothelial cell proliferation, migration, and tube formation on matrigel substrates. In addition, we created ischemia in the hind limb of C57BL/6 mice by ligating the femoral artery and delivered FGF-2 or glypisomes with FGF-2 from implanted alginate beads. We monitored the recovery of perfusion in the ischemic hind limb for 14 days using laser speckle. On day 14 the animals were sacrificed and the thigh and calf muscle tissue was isolated and process for paraffin sections. The sections were H&E stained to assess ischemic changes in the muscle fibers and immunostained for PECAM to quantify neovascularization.

Results: Endothelial cells treated with FGF-2 and glypisomes exhibited a 1.5-fold increase in number of tubes formed and 2-fold increase in the number of branching points compared to those treated with FGF-2 alone in the tube formation assay. Endothelial cells treated with VEGF and glypisomes exhibited a similar trend verses those treated only with VEGF. Mice treated with FGF-2 and glypisomes exhibited a significant increase in relative blood flow compared to mice treated with just FGF-2 alone 7 and 14 days post injury (**Figure 1A**). Histological analysis showed that mice treated with FGF-2 and glypisomes had significantly less damaged muscle fibers in both the calf and the thigh than those treated with FGF-2 alone (**Figure 1B**). Co-delivery of FGF-2 and glypisomes also led to a significant increase in small vessels in both the calf and thigh (**Figure 1C**) as well as a significant increase in large vessel density in the thigh muscle of mice compared to those treated with FGF-2 only.

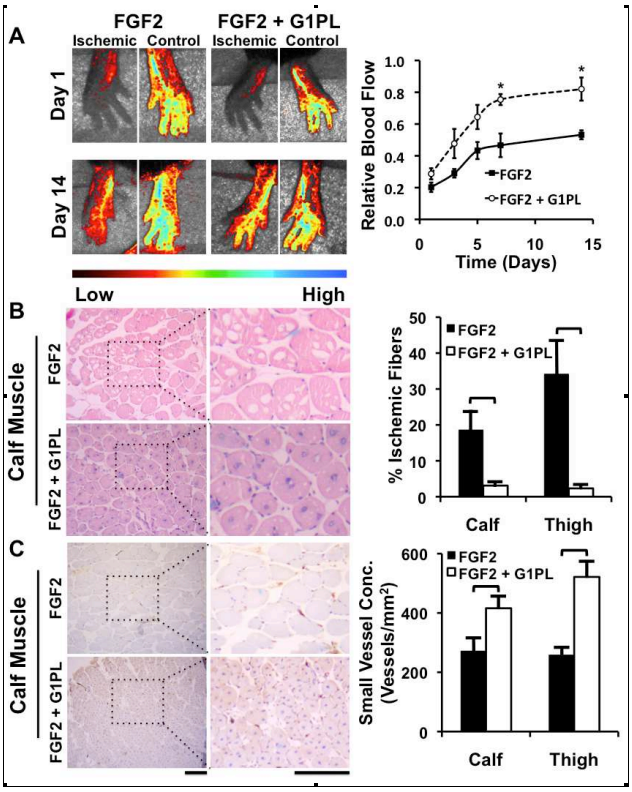

Figure 1. (A) Relative blood flow with respect to contralateral limb and relative blood flow measurements from laser speckle imaging. (B) H&E sections showing damaged muscle fibers, and graph of the percent of fibers affected by ischemic damage in the calf and thigh muscle. (C) PECAM stained sections and small vessel density in calf and thigh muscle. A * or brackets indicate statistically significant differences. Bar =100 µm.

Conclusions: Glypisomes significantly enhance the effect of FGF-2 on cultured endothelial cells leading to increased proliferation, migration and angiogenic differentiation. In addition, glypisomes delivered in combination with FGF-2 from an alginate gel increase revascularization of ischemia hind limb in mice. Thus, locally delivered growth factors in combination glypisomes may be a promising approach for treating peripheral vascular disease.