Pro-Angiogenic Cascade Signaling as the Mechanism for Vascular Regenerative Medicine

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Introduction: New therapies to afford limb salvage in patients with imminent amputation due to critical limb ischemia are needed. We have previously shown E-selectin as an essential participant in neovascularization. Thus, we hypothesized E-selectin supercharged mesenchymal stem cells (E-selectin$^+$/MSC) would augment limb reperfusion, tissue regeneration, and functionality.

Methods: C57Bl6 mice underwent femoral artery ligation and received either vehicle (PBS, n=9) or syngeneic donor MSCs, transduced *ex vivo* to express either GFP$^+$ (control, n=20) or E-selectin-GFP$^+$ (treatment, n=18). Laser doppler imaging (LDI), confocal laser microscopy, and treadmill exhaustion test were utilized to determine neovascularization and limb function. Extent of atrophy (myocyte size, μm^2) was assessed via histology of leg muscles. RT^2 Profiler PCR Array analysis of 84 genes involved in angiogenesis to assess therapeutic mechanism of action. Student's t-test or ANOVA was utilized to compare means and significance set at $p<0.05$.

Results: Compared with GFP$^+$/MSC and PBS, treatment with E-selectin-GFP$^+$/MSC increased ischemic leg LDI reperfusion (55% vs. 39% vs. 24%, $p<0.001$), ischemic mouse footpad vessel density (23% vs. 14% vs. 14%, $p<0.01$) and treadmill distance traversed (162m vs. 111m vs. 110m, $p<0.01$). The ligated limb in mice treated with E-selectin-GFP$^+$/MSC were less atrophic than controls ($793\mu m^2$ vs. $556\mu m^2$ vs. $546\mu m^2$, $p<0.001$). Seven pro-angiogenic genes was upregulated in E-selectin-GFP$^+$/MSC treated ischemic leg tissue while tumor necrosis factor (TNF) was downregulated, when compared with GFP$^+$/MSC treated tissues.

Conclusion: This innovative E-selectin supercharged stem cell therapy confers increased limb reperfusion, function, and decreased atrophy, likely via upregulation of pro-angiogenic cytokines and downregulated TNF.

Table 1. Changes in relative expression of genes between E-selectin$^+$/MSC vs GFP$^+$/MSC and E-selectin$^+$/MSC tissue *vs* GFP$^+$/MSC tissue

MSC-Treated Ischemic Leg Tissue Fold Change (E-selectin$^+$/GFP$^+$)	Gene	MSC Fold Change (E-selectin$^+$/GFP$^+$)	Gene
2	Csf3*	10.5	Cxcl2**
2	Cxcl5*	2	F2**
2	Serpine1*	2.5	Lep**
-3	TNF	2.3	Tbx1**

* genes only upregulated in MSC-treated ischemic leg tissue, ** genes upregulated in MSC and MSC-treated ischemic leg tissue, Csf3: Colony stimulating factor 3 (granulocyte), Cxcl5: Chemokine (C-X-C motif) ligand 5, Serpine1: Serine (or cysteine) peptidase inhibitor, clade E, member, TNF: tumor necrosis factor, Cxcl2: Chemokine (C-X-C motif) ligand 2, F2: Coagulation factor II, Lep: Leptin, Tbx1: T-box 1