

Association of hASCs with Murine Retinal Vasculature after Intravitreal Delivery

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Introduction Diabetic retinopathy results from structural abnormalities of retinal vasculature. Loss of vascular integrity results in macular edema and exudation, and retinal and vitreous hemorrhage. An ideal strategy for intervention would be to restore structural integrity of damaged vessels, as well as mature the compensatory neovascularization that occurs in this disease. Bone marrow-derived stem cells injected intravitreally have been shown to incorporate into damaged retinal vessels in murine models of diabetic retinopathy through reendothelialization of acellular vessels. The initial insult in diabetic retinopathy is a loss of pericyte support cells on existing vessels and a subsequent lack of pericytes on immature neovascularization. Recently published work from several labs has demonstrated hASCs can assume a pericyte-like role, associating abluminally with vessels while also expressing pericyte molecular markers such as NG2. The use of human adipose-derived stem cells (ASCs) for treating retinal disease has not yet been investigated. Specifically, the ability of hASCs injected intravitreally to cross the internal limiting membrane (ILM) of the retina and subsequently associate with retinal vasculature is unknown. In this study, we examine the ability of intravitreally-injected hASCs to penetrate the ILM and physically associate with blood vessels of a murine retinal vascular network.

Methods CD-1 (n = 6) and NIH-III (n = 2) immunocompromised 8-week-old female mice received intravitreal injections of DiI-labeled hASCs using a 30 gauge Hamilton syringe through the pars plana. Passage 3 cells were injected in 2 ul sterile PBS, with each eye receiving a different concentration of hASCs (25 million cells or 12.5 million cells/mL). Two or ten days post-injection, retinas were harvested, whole-mounted, and immunostained to label blood vessels. Retinal vascular networks were stained with isolectin and TOTO nuclear stain and imaged using confocal microscopy.

Results Two days after hASC intravitreal injection, individual hASCs as well as aggregated masses of hASCs were present above the superficial vascular network, but penetration of the ILM was not detected, as there were no apparent instances of coplanarity between hASCs and retinal vessels. After ten days, hASCs were observed in the vascular bed both in aggregated masses and discrete cellular units. hASCs in the vascular bed did not directly associate with blood vessels, and no quantifiable differences in vascular length density were observed in the retina as compared to sham-injected controls. Many hASCs stained positive for isolectin, a stain that also labels endothelial cells. When hASCs were present in an aggregated mass, lectin staining was almost exclusively limited to cells on the surface periphery of the mass.

Conclusion We have shown that hASCs, when injected into the murine ocular vitreous, have the ability to cross the ILM and reach the vascular bed of the retina by ten days post-injection. As a result, it may be possible to utilize hASCs as a therapeutic strategy to target damaged retinal vessels. Our observations may indicate that in the nondiabetic retinal compartment, hASCs will assume an extravascular endothelial phenotype instead of a perivascular phenotype. The phenotype of injected hASCs in a murine diabetic model remains to be determined.

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