Endothelial Colony Forming Cells Retain their Vessel Forming Capacity Following Lentiviral Transduction

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Summary: We assessed the effects of lentiviral transduction of two optically traceable proteins, eGFP and luciferase, on the vessel forming capacity of endothelial colony forming cells.

The transplantation of vessel forming cells is a promising strategy to promote neovascularization and collateral vessel formation in vivo. Endothelial colony forming cells (ECFCs) are a vessel forming cell population that can be isolated from both cord and peripheral blood and possess superior proangiogenic properties when compared to human microvascular endothelial cells (HMVECs). Genetically modified ECFCs designed to overexpress key angiogenic or optically traceable proteins may be a useful tool in further promoting vasculogenesis in vivo, as well as in tracking the presence of these cells following implantation. In order to capitalize on this potential, we examined the impact of lentiviral transduction of two marker proteins, enhanced green fluorescent protein (eGFP) and luciferase, on the vessel forming capacity of ECFCs using in vitro and in vivo models.

a) b) The proliferative capacity of eGFP-transduced ECFCs (MOI=10) was similar to control cells and greater than HMVECs (p < 0.05).

ECFCs were transduced with eGFP at varying multiplicity of infection (MOI) using a pCCL-CMV-GFP VSV pseudotyped HIV-1 derived lentiviral virus and sorted to exclude nontransduced cells (Fig. 1a). The angiogenic potential of both lentiviral-transduced (eGFP) and control ECFCs were compared to HMVECs using a variety of in vitro assays including cell proliferation, migration, and tube formation. Luciferase-transduced ECFCs were then characterized for luminescence both in vitro and in vivo.

We detected no significant differences in the proliferation of eGFP-transduced ECFCs prepared at MOI=10 compared to control ECFCs (MOI=0) (Fig. 1b). ECFCs transduced at an MOI of 100 displayed a reduced proliferative capacity while those transduced at MOI=1 exhibited the weakest eGFP expression. ECFCs transduced at an MOI=10 also displayed robust eGFP expression, while showing little to no reduction in their capacity to migrate in response to chemotactic gradients, uptake acetylated LDL, or form tube-like structures when cultured on Matrigel. ECFCs transduced with luciferase (MOI=10) showed a linear correlation between cell number and luminescence when observed in vitro. These cells were also detected in vivo for 2 weeks in a rat calvarial defect model using a Xenogen IVIS optical imaging system.

Genetic modification of transplantable cell populations requires an optimization of the balance between transduced protein production and the maintenance of normal cell phenotype. In this study, we determined that lentiviral-transduced ECFCs, a promising cell population in the field of neovascularization, maintained their vessel-forming capacity while also producing efficacious amounts of optically traceable proteins.