

Characterization of ASC Migration Toward a Physiological Concentration of PDGF-BB

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INTRODUCTION: Several recent studies have hinted at the ability of human adipose-derived stem cells (ASCs) to behave in a similar fashion to perivascular cells (pericytes or smooth muscle cells). While transcription of RNA for PDGFbeta receptor and production of RNA coding for PDGF-BB suggest a responsiveness to perivascular guidance cues, the functional chemotactic effect of physiological concentrations of PDGF-BB has not been examined for this cell type. If ASCs were to play a perivascular role *in vivo*, they would first need to respond to perivascular guidance cues such as PDGF-BB. In this model, migration of ASCs through a Boyden chamber system in response to differential presentation of PDGF-BB is measured in order to elucidate the potential perivascular functionality of the cell type.

METHODS: Dil-labeled ASCs were cultured in DMEM/F12 with 3% FBS for 48 hours in order to induce quiescence before being seeded onto a Transwell insert membrane. 24 hours before the migration experiment, half of the cells were placed in a hypoxic environment using a Modular Incubation Chamber purged of oxygen with 5% CO₂, 95% N₂ gas mixture. A suspension of ASCs at passage 6 in DMEM/F12 with 10% FBS was used to deliver cells to the upper surface of the membrane at a concentration of 50 kcells/cm². The lower chamber of the Transwell migration system was filled with DMEM/F12 with 10% FBS containing 1 ng/mL human PDGF-BB, 1 ng/mL human PDGF-BB plus 2.5 ug/mL of PDGFbeta receptor antibody, or PBS vehicle. Cells were allowed to migrate for 90 minutes and 3 hours before being fixed with 4% paraformaldehyde. Nuclei were labeled with Hoechst 33258 nuclear stain. Images were then analyzed with a confocal microscope using a 20x objective lens and quantified by counting cells in on both upper and lower membrane surfaces and expressing the quantity migrated by percentage of total cells in each field of view (n=5).

RESULTS: A significant increase in ASC migration after 3 hours was seen in cells exposed to PDGF-BB as compared to those exposed to PBS vehicle, while no such increase was seen in those treated with PDGF-BB and PDGFbeta receptor antibody compared to PBS vehicle. Additionally, significantly more ASCs cultured in hypoxia migrated to the lower compartment of the Transwell system after 90 minutes compared to those presented with PDGF-BB with PDGFbeta receptor antibody.

CONCLUSIONS: These data suggest that ASCs can migrate toward a chemotactic PDGF-BB signal even at low physiological concentrations of growth factor. Furthermore, ASCs appear to suffer no adverse effects from 24 hours of culture in hypoxic conditions.