Elucidating the role of integrin α5 in mediating the therapeutic potency of circulating angiogenic cells cultured on collagen matrix

Brian McNeill, **Branka Vulesevic**, Marc Ruel, Erik J. Suuronen
Division of Cardiac Surgery, University of Ottawa Heart Institute, Ottawa, ON, Canada.

Statement of Purpose: Culturing peripheral blood mononuclear cells (PBMCs) on a collagen-based matrix enhances the expansion and therapeutic potential of circulating angiogenic cells (CACs).[1] Integrins are αβ heterodimeric transmembrane glycoproteins that link the extracellular matrix and a cell's intracellular cytoskeleton. They regulate a number of cellular processes which are likely involved in the response of CACs to the collagen.[2] The aim of this study was to investigate the role of integrin alpha 5 (α5) – a protein that binds fibronectin and laminin – in the pro-angiogenic effect of CACs cultured on a collagen matrix (cmCACs). Both fibronectin and its receptor integrin α5β1 directly regulate angiogenesis, but the effect of collagen matrix on expression and function of this integrin has not yet been examined. The role of α5 was investigated *in vitro* and *in vivo*, including the use of the known α5-interacting proteins angiopoietin-1 and -2.

Methods: By qRT-PCR, the expression of several collagen-binding (α1, α2, α10, α11 and β1) and several pro-angiogenic integrin genes (α5, αV, β3 and β5) were evaluated in human CACs after a 4-day culture on fibronectin or collagen matrix (prepared from collagen I and chondroitin sulfate-C, final concentration of collagen 2.35 mg/ml). Western blot was performed to measure protein levels. Using a specific blocking antibody that targets α5 (Abcam), the role of this protein in cmCAC function was assessed. Specifically, cell phenotype (by flow cytometry), as well as adhesion, migration and angiogenic potential were examined. *In vivo*, a hindlimb ligation CD-1 mouse model was used. Animals were injected 20min after ligation with PBS (PBS control), cmCACs, or cmCACs with blocked α5. Hindlimb perfusion was monitored over time by laser Doppler, and arteriole density in the hindlimb muscle was evaluated by immunocytochemistry at 2 wks. To further investigate the mechanism of α5 pro-angiogenic effects, cmCACs were stimulated with angiopoietins for 2h prior to functional analysis such as adhesion, migration and proliferation.

Results: mRNA and protein levels of integrin α5 were increased in CACs after 4-day culture on collagen matrix vs. fibronectin. The functional importance of this protein was demonstrated by blocking α5 for two hours prior to functional assays. A significant reduction was observed in the ability of α5-blocked cmCACs to adhere to collagen ($p<0.01$), migrate in response to VEGF ($p<0.03$), and incorporate into tubule-like structures formed during an angiogenesis assay ($p<0.04$). *In vivo*, the recovery of blood flow was reduced in mice treated with α5-blocked cmCACs compared to the control cmCACs group at day 7 ($p=0.01$) and day 14 ($p=0.03$) post-ligation (Figure). The number of smooth muscle actin (SMA)-positive blood vessels per field-of-view was also significantly lower in animals treated with α5-blocked cmCACs ($p=0.02$) compared to the cmCACs group. After establishing the essential role of α5 in the function and pro-angiogenic potency of cmCACs, CACs were further stimulated *in vitro* with angiopoietin-1 and -2, added to the culture media. Angiopoietin-1-stimulated cmCACs had a 1.6-fold increase in migration ($p=0.03$) and a 1.8-fold increase in cell incorporation into tube-like vessels in an angiogenesis assay compared to the control cmCACs ($p=0.04$).

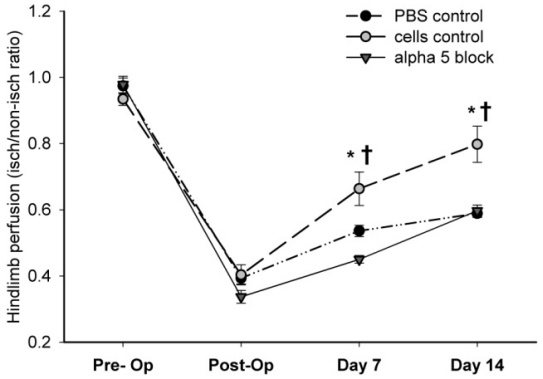

Figure 1. Blood perfusion was restored in the ischemic muscle of mice 2 weeks after treatment with collagen matrix-cultured CACs (cells control), but not with α5-blocked cmCACs. Perfusion was measured by Laser Doppler analysis and data are presented as the average ratio of ischemic-to-non-ischemic hindlimb blood flow (*$p\leq0.02$ for PBS control vs. cells control; †$p=0.03$ for α5 block vs. control cells).

Conclusions: The culture of PBMCs on a collagen matrix enhances their angiogenic potential, at least in part, through the regulation of integrin α5. While α5 is not a collagen-binding protein, it is still affected by collagen matrix, which was seen through mRNA and protein expression. Blocking the activity of α5 in cmCACs reversed the improvements, observed *in vitro* and *in vivo*, in their function and pro-angiogenic effects. This study also presents a possible new way to increase the therapeutic effect of cmCACs by use of angiopoietin-1. Overall, we provide insight into novel mechanisms for improving the function of therapeutic CACs.

References:

[1] Kuraitis D. J Mol Cell Cardiol. 2011; 51(2):187-97.
[2] Semi K. Am J Pathol. 2000 April; 156(4): 1345–1362.