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Effect of TPS, a peptide designed to specifically recruit blood outgrowth endothelial cells, on a novel smooth muscle cell phenotype derived from human blood

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Introduction: Circulating progenitor cells are interesting targets for tissue engineering applications due to their potential for regeneration, repair, and remodelling. Of these cells, blood outgrowth endothelial cells (BOECs) show particular promise, and as such a novel peptide (TPS; TPSLEQRTVYAK) was synthesized to specifically recruit these cells[1]. In this study, we have isolated another cell type from the blood with potential for vessel regeneration, the so-called, blood outgrowth smooth muscle cells (BOSMCs), in which we sought to investigate the potential proliferative effects of TPS. The effects of TPS on stromal cells were measured to establish selectivity of the peptide for cells derived from blood progenitors.

Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from human blood and differentiated using endothelial cell selective media as described previously[2,3]. Cells emerging from 6 donors were, as expected consistent with a BOECs phenotype. However, cells emerging from one donor where blood had hemolysed were characterized as BOSMCs. The phenotype of both cell types was characterized by FACS analysis of smooth muscle alpha (SMA), CD31 (endothelial cell marker), and CD34 (hematopoietic progenitor cell marker). The effect of TPS (1-1000µM) on cell proliferation was studied using Alamarblue assays and CXCL8 release was determined by ELISA. Human aortic valve endothelial cells (hVECs), interstitial cells (hVICs) and A549 were used as negative controls. Data was analysed by one-way ANOVA followed by Dunnett's Multiple Comparison Test.

Results: FACS analysis showed that BOSMCs were positive for SMA, negative for CD31 and expressed relatively low levels of CD34. BOECs were positive for CD31, CD34 and SMA. Proliferation was stimulated by TPS (10,100,1000µM) in BOECs ($p<0.05$, n=6; 1.23, 1.24, and 1.30 folds,respectively) and at 100 and 1000µM in BOSMCs ($p<0.05$, n=4; 1 folds in both)with no effect on hVICs, hVECs, and A549 (n=3). TPS (1000µM) reduced CXCL8 release from BOECs (n=6, 3100.97±800,mean± SEM) with no effect on release from BOSMCs (n=3, 410±160,mean± SEM).

Conclusion: We show for the first time that the BOEC-specific peptide TPS[1] has pro-proliferative effects, without inducing inflammation, on the relatively new cell type, BOSMCs, without affecting proliferation in non-blood progenitor derived cell comparators. This data could be valuable in the field of tissue engineering where vascular smooth muscle and endothelial cell recruitment are both required to generate replacement tissues.

References:

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