Probing cell signaling in endothelial cells stimulated with S1P, VEGF, platelet poor plasma and shear stress

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Statement of Purpose: Endothelial cell migration is important in angiogenesis, vasculogenesis and the endothelialization of materials. Due to the complexity of these processes, we are using knowledge of the activation states of endothelial cells to improve the design of bioactive materials. Previously, we have demonstrated that the chemokinetic factors sphingosine 1-phosphate (S1P) and VEGF act synergistically in static culture, but in the presence of fluid shear stress, either factor alone is sufficient for maximal endothelial cell migration.[1] Similar trends are also found when these factors are added to platelet poor plasma, which is more relevant to *in vivo* applications.

To better understand the complexities of these cellular responses, we are applying sensitivity analysis to determine the elasticities of enzymes in the signaling cascade. This is precisely the information needed to improve the design of bioactive materials. In addition to manipulating environmental conditions, we are also perturbing intracellular components of the cascade. To make this approach feasible, we need large numbers of endothelial cells. We have thus produced hTERT immortalized endothelial cells, which we describe here.

Methods: Endothelial cells were cultured in MCDB 131 medium supplemented with 10 ng/ml epidermal growth factor, 10 µg/mL heparin, 1.0 µg/mL hydrocortisone, 2% fetal bovine serum and 2 mL of 3 mg/mL bovine brain extract. Platelet poor plasma was collected from donors with 2 U/mL heparin. Shear stress was applied with a spinning disk apparatus,[1] or with a gravity driven parallel plate flow chamber. HAEC were immortalized by transduction of hTERT with amphotrophic Moloney murine leukemia virus (MMLV). The mouse receptor for ecotropic MMLV was transduced into the immortalized cells using amphotrophic MMLV.

Results / Discussion: Endothelial cells respond to S1P added to platelet poor plasma. However, unlike in low serum medium,[1] the combination of 10 ng VEGF and 100 nM S1P has some effect on endothelial cell migration in the presence of shear stress (Fig. 1).

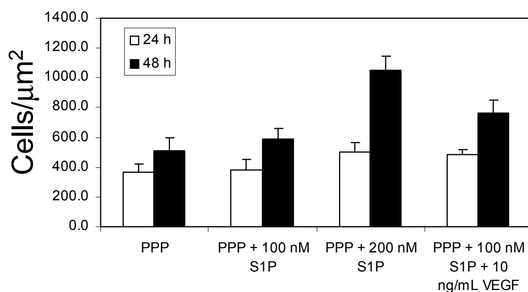

Figure 1: Using a scrape wound assay in the presence of fluid shear stress, migration of HUVEC into a scrape wound was assessed at 24 and 48 h. Cells were exposed to ≈ 2.5 dyne/cm² shear stress using a spinning disc apparatus.

To understand the synergy between these stimuli at the level of cell signaling will require a large number of endothelial cells. Loss of endothelial phenotype in culture can be prevented by stable transduction of hTERT into the endothelial cells.[2] We demonstrate here that migration of hTERT-expressing HAEC in the presence of S1P is not different from normal HAEC (Fig. 2). These cells were further transduced with a mouse receptor that is targeted by ecotropic Moloney murine leukemia virus. Human cells expressing this receptor could be infected with this virus more safely than with retroviruses that infect all human cells.

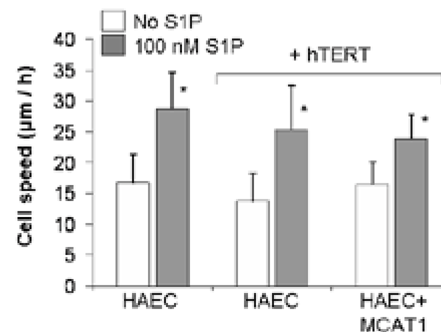

Figure 2: Using a gravity-driven flow chamber, human aortic endothelial cells (HAEC) were exposed to 20 dyne/cm² shear stress for 12 h. Cell migration speeds were determined by time-lapse microscopy. Migration response to S1P was similar for HAEC, HAEC transduced with hTERT, and HAEC-hT cells additionally transduced with the mouse cationic amino acid transporter 1. Asterisks indicate $p < 0.05$ versus conditions without added S1P.

Using these immortalized cells, we will produce endothelial cell lines with stable knockdowns of the main S1P receptor ($S1P_1$). We will also increase the activities of Akt and Rac by knockdown of the highly specific Rac-GAP β_2-chimaerin and the Akt phosphatase PHLPP. The response of these cell lines to S1P, VEGF, shear stress and platelet poor plasma should demonstrate where the S1P signaling cascade that leads to cell migration becomes saturated.

Conclusions: The study of cell signaling is difficult in primary cells that have limited replicative capacity. Here, we illustrate that immortalization of cells may aid in producing cell lines that exhibit normal migration responses but which can be readily manipulated at the intracellular level.

References:

1. Hughes et al., ABME 33 (2005) 1003-1014
2. Freedman & Folkman, Cell Cycle 3 (2004) 811