Endothelial Cell Migration Response to Angiogenic and Osteogenic Growth Factors

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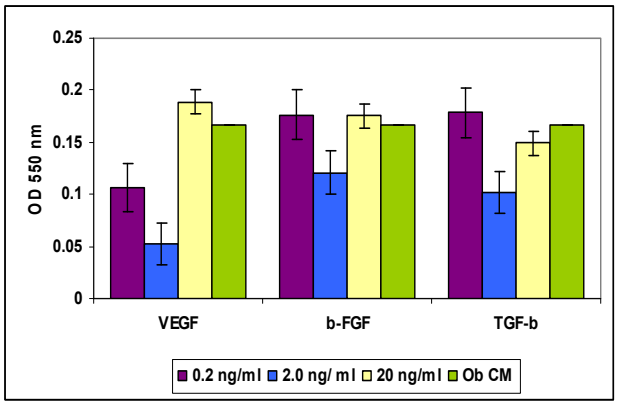
Statement of Purpose: Vascular endothelial growth factor (VEGF) is a potent mitogen and chemoattractant for endothelial cells. They are directed from an established vessel by a progressive VEGF concentration gradient toward the wound bed. This growth factor is also produced and used by other cell types. Of particular interest is its production and use by osteoblasts. The use of this growth factor by both endothelial cells and osteoblasts suggests perhaps a reciprocal relationship between these cell types modulated by a common growth factor. Osteoblasts also produce several other factors that have angiogenic effects. Two are transforming growth factor beta-1 (TGF-β_1), and basic fibroblast growth factor (b-FGF). The chemoattractant effects of these growth factors on endothelial cells, however, are not clear. In this study we use an assay based on the Boyden chamber principle to evaluate the chemoattractant effects of three concentrations of VEGF, TGF-β_1 and b-FGF on endothelial cells. Media from cultured human fetal osteoblasts was taken and evaluated as well.

Methods: Human umbilical vein endothelial cells (HUVECs) were aseptically isolated from fresh umbilical cords (University Hospital, San Antonio, TX), plated, and cultured to passage three or four[1]. Cells were maintained in complete medium as follows: M199 with Hank's salts and sodium bicarbonate supplemented with 2 mM L-glutamine, 25 mM HEPES, 10% fetal bovine serum (FBS), 150 U/mL penicillin- streptomycin solution, 30 µg/mL endothelial cell growth supplement (ECGS), and 10 µg/mL heparin (all from Sigma Aldrich, St. Louis, MO). HUVECs were grown in a 37 °C incubator with 5% carbon dioxide. Human fetal osteoblasts were purchased from the American Type Culture Collection (ATCC®, Manassas, VA) and maintained in a media containing: 1:1 mixture of Ham's F12 medium and Dulbecco's Modified Eagle's Medium (DMEM) without phenol red and supplemented with 2.5 mM L-glutamine, 0.3 mg/mL G418, and 10% FBS (all from Sigma Aldrich, St. Louis, MO). Cells were grown in a 34 °C incubator with 5% carbon dioxide. Vascular endothelial growth factor (VEGF), Transforming Growth Factor- β_1 (TGF-β_1), and basic fibroblast growth factor (b-FGF) were all from Sigma Aldrich (St. Louis, MO).

Chemicon® International's (Temecula, CA) QCM™ 24-Well Colormetric Cell Migration Assay is based on the Boyden chamber principle and was used to evaluate HUVEC migration in response to three concentrations of each growth factor. Specifically, a kit containing polycarbonate membrane inserts with 8 µm pores was selected and 200,000 HUVECs suspended in 300 µL of complete media were seeded inside the inserts. Inserts were placed in the well of a 24 well plate containing either 0.2 ng/mL, 2 ng/mL, or 20 ng/mL of growth factor in complete media; or media from cultured human fetal osteoblasts (conditioned media- ObCM). Plates were covered and incubated at 37 °C in a 5% CO_2 incubator overnight. Cells remaining in the insert were removed and migrated cells were stained on the membrane. The inserts were allowed to air dry then the stain was extracted and 100 µL was transferred to a 96 well plate. The plate was read on a microplate reader (Bio-Rad, Richmond, CA) at 550 nm. Data was analyzed with a one way analysis of variance (ANOVA).

Results:



At the two lowest concentrations of growth factor, VEGF was not as powerful a chemoattractant as either b-FGF or TGF-β_1. There was no statistical difference however, between b-FGF and TGF-β_1 at these concentrations. At the lowest concentration ObCM was just as potent as b-FGF and TGF-β_1 and at the median concentration ObCM was statistically more potent than all growth factors tested. At the highest concentration VEGF was a statistically more effective chemoattractant than the other growth factors and insignificantly more effective than ObCM. Each growth factor displayed a bi-phasic pattern of potency stimulating more cells to migrate at very low and very high concentrations (Figure 1).

Conclusion: While each growth factor at each concentration successfully stimulated chemotaxis, in an *in vivo* system these cells are exposed to several growth factors of concentrations that vary over time. To attract these cells to a site where they do not already exist, the non-endothelial cells will produce a chemoattractant in the presence of several other factors needed for other functions. The similar capability of VEGF and ObCM to attract cells suggests that VEGF can mimic the complex milieu of an *in vivo* system thus allowing simplified *in vitro* analysis of endothelial cell behavior.

References: 1. Freshney RI. Endothelium. Culture of Animal Cells. Hoboken: John Wiley & Sons, Inc.; 2005. p 404- 406.