Specific Role of Endomucin in Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) Internalization and Function

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Endomucin (EMCN) is a type I integral membrane glycoprotein selectively expressed by endothelial cells in venous and capillary. We have previously showed that EMCN knockdown significantly inhibits VEGF165-induced VEGFR2 internalization and endothelial cell migration, proliferation, and tube formation. The goal of this study is to further define the specificity of EMCN for the VEGF/VEGFR2 system by determining the role of EMCN in VEGF121-induced VEGFR2 activation and migration, VEGF165-induced VEGFR1 internalization, as well as fibroblast growth factor (FGF)-induced cell migration and receptor internalization. EMCN was knocked down in human retinal endothelial cells (HRECs) using siEMCN, with non-targeting siRNA as a control. siEMCN significantly reduced EMCN protein levels compared to the non-targeting siRNA group by 95% ($P<0.05$). Endothelial cells (EC) migration was assessed in a scratch wound healing assay. VEGF165, VEGF121 and FGF stimulation significantly increased HRECs wound closure compared to control (1 ± 0.02 vs. 1.15 ± 0.02, $p=0.004$; 1 ± 0.02 vs. 1.18 ± 0.03, $p<0.0001$. 1 ± 0.03 vs. 1.25 ± 0.04, $p<0.0001$; $n>3$ for all groups). EMCN knockdown prevented HRECs migration induced by VEGF165 (1 ± 0.03 vs. 1.04 ± 0.03, $p=0.9$, $n=3$) and VEGF121 (1 ± 0.03 vs. 1.07 ± 0.02, $p>0.05$, $n=3$), but not FGF induced migration (1 ± 0.03 vs. 1.18 ± 0.05, $p<0.0001$, $n=6$), compared to control. Receptor internalization was examined by cell surface biotinylation assay and quantified by Western blot. EMCN depletion prevented VEGF-165 induced VEGFR2 internalization (0.73 ± 0.32 vs. 0.71 ± 0.29, $p=0.74$, $n=7$) but did not impact VEGFR1 (1.50 ± 0.12 vs. 0.73 ± 0.11, $p<0.001$, $n=6$) or FGF-induced FGFR1 internalization (1.03 ± 0.16 vs. 0.73 ± 0.12, $p<0.05$, $n=7$). We conclude that EMCN is essential for VEGF165- and VEGF121-induced EC migration and VEGR2 internalization. However, EMCN does not play a significant role in VEGFR1 internalization or FGF-induced internalization and endothelial cells migration. Our data indicate a specific role for EMCN in the VEGF/VEGFR2 system.