Extracellular ligand immobilization alters vascular morphogenesis by changing intracellular trafficking of receptors: evidence from a multi-scale computational model

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Abstract

Recent studies have demonstrated that vascular endothelial growth factor (VEGF) isoforms that are bound to the extracellular matrix can also ligate and activate the cell surface receptor VEGFR2. However, the resultant vascular networks are structurally and functionally different than those formed after stimulation with solely soluble forms of VEGF. While the majority of VEGF in tissues is predicted to be bound to the extracellular matrix, the mechanism by which this immobilized VEGF differentially activates VEGFR2 is not well understood. We have developed a multi-scale, experimentally-validated computational model of VEGFR2 ligation, intracellular trafficking, and site-specific phosphorylation in response to soluble and immobilized VEGF *in vitro*. The model includes the extracellular space, cell surface, and two distinct types of endosomes within endothelial cells. With this model, we show that VEGF immobilization leads to decreased internalization of VEGFR2 (due to scaffolding of VEGF to the extracellular matrix), altering the internal-to-surface distribution of VEGFR2, and in turn shifting the balance of VEGFR2 phosphorylation on tyrosines 1175 and 1214, which lead to cell proliferation and migration respectively. We show that altered VEGFR2 trafficking due to VEGF immobilization is sufficient to mechanistically explain all available data on VEGFR2 phosphorylation after stimulation with immobilized VEGF, and suggests that the receptors do not undergo different conformational changes in response to matrix-bound VEGF. Additionally, our model correctly predicts the impact on multiple VEGFR2 phosphotyrosines of changes in VEGFR2 trafficking, interactions with the coreceptor Neuropilin-1 (NRP1), and the expression or activity of phosphatases acting on VEGFR2. Here, we extend this cell-level model to a whole-body framework to study the implications of matrix-bound VEGF *in vivo*, using a compartmental model with multiple tissues comprising parenchymal cells, endothelial cells, extracellular space and proteins specific to those tissues. We include two membrane-resident VEGF receptors (VEGFR1 & VEGFR2) and one coreceptor (NRP1) on endothelial cells, one soluble receptor (sVEGFR1), four isoforms of VEGF (VEGFA165, VEGFA165b, VEGFA121, & VEGFA189) and two isoforms of placental growth factor (PlGF1 & PlGF2). These six VEGF and PlGF ligands have different extracellular matrix-binding and NRP1-binding properties. We examine the influence of the varying NRP1- and matrix-binding properties of the VEGF and PlGF isoforms on whole-body ligand distribution and site-specific activation of VEGFR2 within tissues. We can extend these results to predict the impact of each ligand on vascular remodeling in healthy and diseased tissues. The model is also druggable, allowing us to predict responses to local and systemic delivery of VEGF ligands by protein or gene delivery.