Quantitative Experimental Characterization of Angiogenic Receptors for Systems Biology

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Introduction: Vascular endothelial factor receptor (VEGFR) cell surface localization plays a critical role in transducing VEGF signaling towards pro- and anti-angiogenic outcomes and quantitative characterization of these parameters is critical to advancing computational models, and design and optimization of pro- and anti-angiogenic drugs. However the in vivo levels of these receptors are currently unknown. Therefore, our aims are to quantitatively analyze VEGFR localization on endothelial cells from (a) mouse hindlimb skeletal muscles under normal conditions (b) following induction of hindlimb ischemia, a model for human peripheral artery disease, and (c) breast cancer xenografts.
Materials and Methods: Flow cytometry is used to measure and compare the in vivo surface localization of VEGFR1 and VEGFR2 on endothelial cells isolated from C57BL/6 and BALB/c gastrocnemius and tibialis anterior hindlimb muscles. Endothelial cells are isolated from C57BL/6 and BALB/c normal adult mice, C57BL/6 mice 3 and 10 days after unilateral ligation of the femoral artery, and athymic nude mice 3 and 6 weeks after inoculation with MDA-MB-231 cells.
Results and Discussion: (a) Skeletal Muscle. The data show a 2-fold higher VEGFR1 surface-localization relative to VEGFR2 in endothelial cells from normal mice with 2,000-3,700 VEGFR1/endothelial cell and 1,300-2,000 VEGFR2/endothelial cell. We determine that the glycolytic muscle, tibialis anterior, contains a 30% higher VEGFR1 surface-localization than gastrocnemius, a tissue containing both glycolytic and oxidative fibers. Applying cell-by-cell analysis revealed that these ensemble averaged differences in receptor surface-localization are attributed to a population of endothelial cells presenting low-numbers of VEGFR2. Our results demonstrate that only small VEGFR localization differences exist across the strains, with BALB/c mice displaying a ~17% higher VEGFR1 surface-localization than C57BL/6. **(b) Hindlimb Ischemia.** We determine that 3 days after hindlimb ischemia VEGFR2 surface-levels are decreased by 80% compared to endothelial cells from the non-ischemic limb, and 10 days after ischemia, we observe a 2-fold increase in the surface-levels of the modulatory receptor, VEGFR1, compared to the non-ischemic limb. The significant downregulation of VEGFR2 and later upregulation of VEGFR1 surface-density indicates that VEGFR1 plays a critical role in the ischemia-induced perfusion-recovery process, a process that includes both angiogenesis and arteriogenesis. **(c) Triple-negative MDA-MB-231 Breast Cancer Xenografts.** We observe robust VEGFR1 and VEGFR2 surface-expression on both tumor cells and endothelial cells from tumor xenografts using Nude mice with 8,200-15,000 VEGFR1/endothelial cell, 1,200-1,700 VEGFR2/endothelial cell, 2,000-2,200 VEGFR1/tumor cell, and ~1,000 VEGFR2/tumor cell.
Conclusions: Identifying the balance of angiogenic receptors is critical to characterizing the vascular microenvironment in angiogenesis-related diseases and to advancing computational models of angiogenesis. These studies reveal significant cell-to-cell heterogeneity in receptor localization, and both ischemia and tumor angiogenesis-induced changes in receptor levels. The quantification of these dissimilarities for the first time, in vivo, provides insight into the balance of modulatory (VEGFR1) and pro-angiogenic (VEGFR2) receptors under normal and pathological conditions and lays a foundation for systems biology approaches to angiogenesis in health and disease.

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