ENDOTHELIAL BARRIER DISRUPTION BY VEGF-MEDIATED SRC ACTIVITY POTENTIATES TUMOR CELL EXTRAVASATION AND METASTASIS

Sara Weis, Jianhua Cui, Leo Barnes, and David Cheresh*
Department of Immunology, The Scripps Research Institute
La Jolla CA 92037, USA
*cheresh@scripps.edu

INTRODUCTION. Tumor cell extravasation is a critical last step in the metastatic cascade, and yet how tumor cells extravasate remains a mystery. Tumor cell growth and metastasis has been linked to expression of VEGF, an angiogenic growth factor that promotes vascular permeability (VP). VEGF induces a Src kinase signaling pathway in endothelial cells that is critical for VEGF-mediated VP, since mice deficient in pp60Src show no VEGF-mediated VP response. Here, we demonstrate that Src-deficient mice are resistant to tumor cell extravasation and metastatic disease since circulating tumor cells are unable to induce VP and thereby breach the endothelial barrier in these animals. Moreover, pharmacological inhibitors of either VEGF, its receptor Flk, or Src kinases block tumor cell extravasation and metastasis in wild type mice. Therefore, disrupting Src signaling preserves host endothelial barrier function, providing a novel host-targeted approach to control metastatic disease.

METHOD. Murine colon or lung tumor cells were injected into the circulation of wildtype or Src-deficient mice, and pulmonary and hepatic metastases was assessed. Alternatively, wildtype mice injected with these tumor lines were first treated with pharmacological inhibitors of VEGF, its receptor Flk, or Src kinases. To assess the role that VEGF plays in endothelial cell barrier function, VE-cadherin, an endothelial cell specific junction protein, was isolated from the lungs of wildtype or Src-deficient mice after intravenous injection with VEGF.

RESULTS. We found a dramatic reduction in tumor cell extravasation and metastases in lungs or livers of mice of mice lacking Src or Yes but not Fyn (Figure 1). However, these animals showed no difference in their ability to support primary tumor growth, suggesting that loss of Src kinase led to a selective effect on tumor cell extravasation and metastasis. At the molecular level, VEGF compromises the endothelial barrier by disrupting a VE-cadherin/β-catenin complex in lung endothelium from wildtype, but not Src-deficient mice. Disrupting the endothelial barrier directly with anti-VE-cadherin both amplifies metastasis in normal mice and overcomes the genetic resistance in Src-deficient mice. Pharmacological blockade of VEGF, VEGFR-2 or Src stabilizes endothelial barrier function and suppresses tumor cell extravasation in vivo.

DISCUSSION. Previous studies have implicated VEGF (1), Flk (2), or Src (3) in tumor growth and metastatic disease. In particular, blockade of tumor cell-associated Src activity has been associated with reduced metastasis (4), but this has been attributed to the requirement of Src activity for tumor cell migration and intravasation into the circulation from the primary tumor site. We demonstrate for the first time that regardless
of the invasive capacity of individual tumor cells, metastatic disease critically depends on the ability of circulating tumor cells to breach the endothelial barrier. We propose that metastatic tumor cells which express and release high levels of VEGF have the capacity to dysregulate at least one critical endothelial cell-cell junctional complex facilitating their extravasation (5). These findings suggest an unexpected therapeutic role for pharmacological inhibitors of VEGF or Src kinases in blocking a host response important for metastatic disease.

**REFERENCES**