# Slit-Robo: Neuronal guides signal in tumor angiogenesis

**Slit and Roundabout (Robo) are well-characterized for neuron and leukocyte guidance. Their governing roles have now been expanded to control tumor-endothelial cell communication and mediate tumor-induced angiogenesis.**

The microenvironment in tumors is known to induce vascular endothelial cells to form new blood vessels for supporting expansion of the tumor mass (Hanahan and Folkman, 1996). New blood vessels arise from preexisting capillaries or postcapillary venules in tumors, and/or by recruiting endothelial precursor cells from the bone-marrow stem cell pool (Rafii and Lyden, 2003). As an initial step, endothelial cells migrate toward angiogenic stimuli derived from the tumor cells. In spite of the extensive studies on the involvement of angiogenic factors, such as vascular endothelial cell growth factor (VEGF), basic fibroblast growth factor (bFGF), and cell adhesion molecules such as integrins and cadherins (Jain, 2003), tumor-endothelial cell communication signals for directional movement are not well defined. In this issue of *Cancer Cell*, Wang and colleagues uncover a novel mechanism for tumor-induced angiogenesis by demonstrating that tumor cells secrete the Slit protein to attract vascular endothelial cells expressing the cognate receptor Robo (Figure 1). Slit-Robo signaling thus becomes a "must see" to researchers in the field of tumor angiogenesis.

Slit was originally identified in *Drosophila* as an extracellular cue to guide axon pathfinding, to promote axon branching, and to control neuronal migration (Nusslein-Volhard et al., 1984; Rothberg et al., 1988). Mammalian Slit consists of three members, Slit1, Slit2, and Slit3. All are expressed in the nervous system but Slit2 and 3 can also be found on other cell types. The Slit gene encodes a secreted protein possessing multiple putative protein binding motifs, including four leucine-rich repeats (LRRs), nine (seven in *Drosophila*) EGF-like repeats, a laminin G domain, and a C-terminal cysteine-rich knot (Rothberg et al., 1990).

Robo, the receptor for Slit, was also initially isolated from *Drosophila* in a genetic screen for mutations affecting axon pathfinding (Kidd et al., 1998). Four (three in *Drosophila*) Robo genes have thus far been identified in organisms ranging from *Drosophila* and *C. elegans* to mice and humans. Like Slit, all Robo members are primarily expressed in the nervous system, but three of them are found in non-neuronal tissues. Robo is a member of the neural cell adhesion molecule (NCAM) family and is characterized as a single-pass transmembrane receptor with an extracellular region containing five immunoglobulin (Ig) domains and three fibronectin type III repeats and an intracellular tail composed of four conserved motifs (CC0, CC1, CC2, and CC3) (Kidd et al., 1998).

Multiple investigations in the neurobiology and immunology fields have established a primary role for Slit-Robo as guides for neuronal migration and leukocyte trafficking by acting as repellents (Wong et al., 2002). Slit-Robo may also signal for directing the movement of epithelial sheaths (Schimmelpfeng et al., 2001) and for controlling muscle precursor cell migration during *Drosophila* myogenesis (Kramer et al., 2001). Interestingly, the guiding signal of Slit seems switched from a repellent in the early phase of muscle precursor cell migration to an attractant during the late phase of myofiber attachment to the epidermis. The study by Wang et al. in this issue (Wang et al., 2003) has now significantly extended the spectrum of Slit-Robo signaling to the area of tumor angiogenesis. In tumors, Slit-Robo signals work as attractant rather than repellent.

In tumor development and metastasis, infiltration of endothelial cells into tumor mass, induction of vessel formation, and invasion of tumor cells into blood vessels involve intimate crosstalk between tumor and endothelial cells leading to coordinate cell migration. Researchers working on guidance cues of neuronal migration have hypothesized that extracellular molecules, such as netrins, semaphorins, ephrins, and the Slits, may also function in mediating tumor angiogenesis and tumor metastasis because (1) DCC (Deleted in Colorectal Cancer), Neuropilin, and Ephs, the receptors for netrins, semaphorins, and ephrins, respectively, are expressed on some tumors and endothelial cells; and (2) Slit-Robo, which seems to be a conserved guiding signal for cells of distinct types, has recently been detected in human prostate tumors (Latil et al., 2003). Wang and collaborators investigated the potential involvement of Slit-Robo signaling in tumor angiogenesis. They demonstrated expression of Slit2 in a broad spectrum of tumor cell lines, such as A375 (human melanoma), SCaBER (bladder squamous carcinoma), SK-N-SH (neuroblastoma), NCI-H446 (small cell lung cancer), T24 (transitional cell carcinoma of urinary bladder), LoVo (colon adeno-

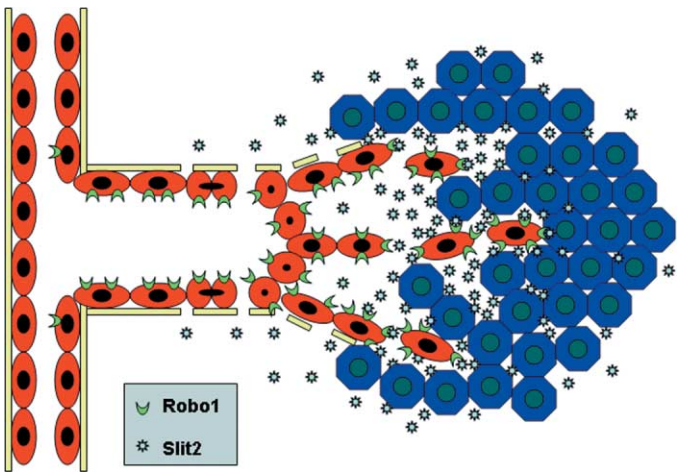


**Figure 1.** Tumor induces angiogenesis through Slit-Robo signaling

Tumor cells secrete the Slit2 protein which forms a gradient field for the attraction of endothelial cells through interaction with Robo1 on endothelial cell surfaces. Endothelial cells migrate toward tumor mass and form new blood vessels. Tumor and endothelial cells are represented by blue and red, respectively.

carcinoma), ZR-75-30 (breast cancer), CNE (nasopharyngeal carcinoma), SMMC-7721 (hepatocellular carcinoma), Acc (adenoid cystic carcinoma of salivary gland), and A673 (rhabdomyosarcoma), as well as a variety of primary tumors, including human melanoma, rectal mucinous adenocarcinoma, invasive breast carcinoma, gastric squamous carcinoma, and hepatocellular carcinoma. Intriguingly, the expression levels of Slit2 in primary tumors appear to correlate with both microvessel density and extent of cancerous alterations, supporting its critical role for tumorigenesis. There appears to be a gradient of Slit2 protein expression in tumors with higher concentrations near the center of the tumor and decreased levels in the periphery, thus creating a signaling gradient field for the attraction of endothelial cells. The team showed the presence of Robo1 on HUVECs (human umbilical vein endothelial cells) and, more importantly, on a melanoma grown in immunodeficient mice. It remains unclear whether expression of Robo1 or other Robo family members is universally presented on the vasculature of other solid tumors that secrete Slit2. Attraction of endothelial cells by Slit2 is mediated through direct interaction with Robo1. Like its role in guidance of cellular movement of neurons and leukocytes, Slit2 controls directional rather than random migration of endothelial cells. Tumor-derived Slit2 seems to specifically attract endothelial cells and does not affect immune cells since there is no difference in the numbers of inflammatory cells inside and outside of the A375-derived melanoma. However, studies in an immunocompetent host will have to confirm such specificity.

Participation of Slit2 in regulating angiogenesis has first been shown by its ability in inducing vascular network formation of HUVECs in vitro. The angiogenic activity of Slit2 is dose dependent and can be inhibited by its antagonist, RoboN, a soluble form of Robo, or by blocking antibodies. A critical role for Slit-Robo signaling in mediating tumor-induced angiogenesis has been further demonstrated in the xenograft melanoma model. Both the density of microvascular vessels in the tumor tissue and the size of the tumor mass were dependent on Slit2 signaling. Tumor size was enlarged if the tumor cells overexpress Slit2 while decreased if soluble RoboN was introduced. It remains to be investigated whether the Slit2-Robo1 interaction is unique or whether redundancy exists. Blocking Slit-Robo binding does not affect normal vasculogenesis since the vascular phenotype appears normal in slit1, $2^{-/-}$ mice (Plump et al., 2002). However, a conclusion cannot be made unless all members of Slit or Robo families have been deleted. It would be interesting to see whether Slit-Robo are involved in tumor metastasis or function in other biological processes such as wound healing or rheumatoid arthritis. One would also be curious whether Robo is expressed on endothelial precursor cells which have been known to migrate and integrate into tumor vessels.

Two important yet less investigated issues are the mechanisms that regulate gene expression of Slit-Robo and their downstream signaling networks in endothelial cells. In vitro, proinflammatory cytokines, such as TNF-$\alpha$ and IL-1$\beta$ can upregulate expression of Slit2 in A375 melanoma cells. Since expression of Slit2 is observed in tumors, it appears obvious to explore whether expression of Slit is governed by the same genetic alternations that drive malignant conversion or whether hypoxia or angiogenic factors induce its expression. Similarly, because Robo is concomitantly expressed in the tumor vasculature, one should ask whether Slit induces Robo expression. The PI3-K pathway has been implicated in mediating Slit2-Robo1 signaling. Activation of this pathway might explain why Slit-Robo function as attractants in directing endothelial cell movement versus repellents for guidance of migration of neurons and leukocytes. Whether other signaling molecules known to deliver Slit-Robo signaling, such as srGAPs, Cdc42, Abelson (Abl), Enabled (Ena), PTPases, and cyclic nucleotides (for review, see Wong et al., 2002), are also involved in Slit-Robo signaling in tumor angiogenesis and what mechanism determine signal for attraction or repulsion need to be addressed.

Zhao-Jun Liu and
Meenhard Herlyn*

The Wistar Institute, 3601 Spruce Street
Philadelphia, Pennsylvania 19104
*E-mail: herlynm@wistar.upenn.edu

**Selected reading**

Hanahan, D., and Folkman, J. (1996). Cell *86*, 353–364.

Jain, R.K. (2003). Nat. Med. *9*, 685–693.

Kidd, T., Brose, K., Mitchell, K.J., Fetter, R.D., Tessier-Lavigne, M., Goodman, C.S., and Tear, G. (1998). Cell *92*, 205–215.

Kramer, S.G., Kidd, T., Simpson, J.H., and Goodman, C.S. (2001). Science *292*, 737–740.

Latil, A., Chene, L., Cochant-Priollet, B., Mangin, P., Fournier, G., Berthon, P., and Cussenot, O. (2003). Int. J. Cancer *103*, 306–315.

Nusslein-Volhard, C., Wieschaus, K., and Kluding, H. (1984). Rouxs Arch. Dev. Biol. *193*, 267–282.

Plump, A.S., Erskine, L., Sabatier, C., Brose, K., Epstein, C.J., and Goodman, C.S. (2002). Neuron *33*, 219–232.

Rafii, S., and Lyden, D. (2003). Nat. Med. *9*, 702–712.

Rothberg, J.M., Hartley, D.A., Walther, Z., and Artavanis-Tsakonas, S. (1988). Cell *55*, 1047–1059.

Rothberg, J.M., Jacobs, J.R., Goodman, C.S., and Artavanis-Tsakonas, S. (1990). Genes Dev. *4*, 2169–2187.

Schimmelpfeng, K., Gogel, S., and Klambt, C. (2001). Mech. Dev. *106*, 25–36.

Wang, B., Xiao, Y., Ding, B.-B., Zhang, N., Yuan, X.-b., Gui, L., Qian, K.-X., Duan, S., Chen, Z., Rao, Y., and Geng, J.-G. (2003). Cancer Cell *4*, this issue.

Wong, K., Park, H.T., Wu, J.Y., and Rao, Y. (2002). Curr. Opin. Genet. Dev. *12*, 583–591.