Aggressive cancers grow progressively, invade locally, and metastasize through a multistep process that involves a malignant cell and a supportive environment (1). Tumor neovascularization critically contributes to tumor growth (2). Hence, there has been great interest in reducing cancer progression by shutting down the tumor blood supply (3). The antiangiogenic drugs currently approved for cancer treatment include bevacizumab (Avastin), a humanized monoclonal antibody blocking vascular endothelial growth factor (VEGF), and sorafenib (Nexavar) and sunitinib (Sutent), synthetic inhibitors of VEGF receptor signaling (4,5). These VEGF-targeted therapies have shown benefit in certain cancer types, but responses have generally been modest and measured in months of extended cancer survival (6–8). This experience raises many questions regarding the appropriate role of current antiangiogenic therapies in cancer treatment (9). In this issue of the Journal, Gray et al. (10) present exciting results that support a potential new strategy, targeting neuropilin-2.

Neuropilins are nonsignaling transmembrane receptors that are shared by two distinct ligand families, the class 3 semaphorins, which regulate neuronal guidance, and the VEGF proteins, which regulate angiogenesis (11,12). Ligand binding to neuropilins dictates their association with specific signal transducers: semaphorins mediate interactions with plexin A receptors, whereas VEGFs mediate interactions with VEGF receptors. VEGF and Sema3A (one of the semaphorins) have overlapping binding domains on the extracellular portion of neuropilin-1 and, for this reason, can compete with one another for binding (13). Two neuropilins are known, neuropilin-1 and neuropilin-2 that have similar structures but differ in their endothelial expression during development. Neuropilin-1 is expressed mainly in the arterial endothelium, whereas neuropilin-2 is expressed mainly in the venous and lymphatic endothelium (14,15). Neuropilin-1 interacts with heparin-binding isoforms of VEGF-A, -B, -E and placental growth factor; neuropilin-2 interacts with VEGF-A, -C, and -D (11,12). Gene-targeting studies have demonstrated that mice lacking functional neuropilin-1 receptors die during embryogenesis with heart and vascular defects, demonstrating a critical role of neuropilin-1 in vascular development (16–18); mice lacking both neuropilin-1 and neuropilin-2 are more severely affected than mice lacking only neuropilin-1 (16–18). Neuropilin-2–deficient mice develop normally, but they are smaller than wild-type mice and display minor abnormalities in the lymphatic system (15). Interestingly, neuropilin-2–deficient mice also display defective retinal neovascularization in response to ischemia (19). Recent studies have shown that administration of neuropilin-1–neutralizing antibodies to tumor-bearing mice reduced tumor angiogenesis and tumor growth, providing evidence for a role of neuropilin-1 in promoting tumor neovascularization (20).

In most cases, neuropilins are the only VEGF receptors expressed by cancer cells (12), and recent studies have correlated expression of neuropilins with poor cancer prognosis (12,21,22). Besides these descriptive reports, it is known that neuropilins

**Neuropilin-2: A New Molecular Target for Antiangiogenic and Antitumor Strategies**

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expressed by cancer cells can bind VEGF and present VEGF to VEGF receptors on adjacent endothelial cells in a juxtacrine manner (23). By this mechanism, neuropilin-1 may enhance tumor angiogenesis and indirectly promote tumor growth (23). It is also known that neuropilin-1 is not only a cell surface receptor for VEGF and class 3 semaphorins but also a regulator of cell adhesion to cells and to the extracellular matrix (24,25). By modulating adhesion, neuropilin-1 may regulate tumor cell migration and invasion, and this property may underlie the observation that neuropilin-1 helped ovarian cancer cells evade contact inhibition (26).

Much less is currently known about the biological properties of neuropilin-2. The exciting experiments by Gray et al. (10) show that neuropilin-2 plays a critical and direct role in colorectal carcinoma tumor progression and may represent a potential therapeutic target for cancers where it is expressed. The authors found that neuropilin-2 expression is present in human colorectal cancer but not in the normal colonic mucosa. Neurupilin-2 silencing in a human colorectal carcinoma cell line reduced phosphorylation of vascular endothelial growth factor receptor-1 (VEGFR-1) and cell motility, invasion, and survival in vitro, providing evidence for a direct functional role of neuropilin-2 in mediating important properties of colon carcinoma cells. Silencing of neuropilin-2 rendered colon carcinoma cells less tumorigenic upon injection into nude mice: primary tumors were much smaller and metastases were fewer than in controls. Strikingly, by injecting mice intraperitoneally with neuropilin-2 siRNA sequences that had been incorporated into neutral liposomes, the authors were successful at selectively reducing neuropilin-2 expression in human colon carcinoma cells that they had implanted in the livers without affecting the levels of murine neuropilin-2 in the tumor vasculature. Using this approach, the authors found that colorectal carcinoma tumors in the livers of treated mice were significantly smaller than in control mice further substantiating the potential of neuropilin-2 as a therapeutic target for colorectal carcinoma.

As one might expect from groundbreaking work, this study raises many questions for future investigation. An important question is how neuropilin-2 silencing reduces tumor growth. The authors show that the activity of the VEGFR-1/Akt pathway is diminished in the colon cancer cells in which neuropilin-2 has been silenced. But it is not clear what role VEGFR-1 signaling plays in the growth of these cancer cells. Because the colon cancer cell line used here expresses endogenous VEGF-A and VEGF-1, it is possible that VEGF functions as an autocrine growth factor for these cells by acting through VEGFR-1. If so, the reduction of neuropilin-2 may diminish VEGF binding to cells and its autocrine activity. To test for this possibility, it would be interesting to investigate the effects of neuropilin-2 targeting in neuropilin-2-positive cancer cells that express no VEGFRs or that express VEGFR-2 or VEGFR-3 but not VEGFR-1. It would also be important to compare the effects of neuropilin-2 silencing with the effects of VEGFR-1 blockade. In their xenograft model, the authors have carefully excluded a contribution of the tumor vascular endothelium to the antitumor efficacy of neuropilin-2 silencing by using human-specific sequences, which reduced human neuropilin-2 in the human tumor cells but not the mouse neuropilin-2 in the mouse-derived tumor vasculature. However, because neuropilin-2 on the tumor cells can bind VEGF and present it to endothelial cells in a juxtacrine manner, it would be important to know whether a reduction of neuropilin-2 on tumor cells could inhibit angiogenesis in the tumor microenvironment. This approach potentially would target both tumor cells and tumor endothelium for increased efficacy.

Class 3 semaphorins are also promising candidates for modulating neuropilin function. These ligands bind to neuropilins and utilize plexin A receptors as signal transducers. In contrast to VEGF, the class 3 semaphorins, Sema3A and Sema3F, repel endothelial cells, promote their death, and inhibit angiogenesis (27–29). Expression levels of Sema3F were inversely associated with the aggressiveness of lung cancer (30), and Sema3F directly inhibited adhesion of lung cancer cells to the extracellular matrix and reduced tumor vascularization (30,31). This finding raises the possibility that a reduction of neuropilin-2 may abrogate the antiangiogenic activities by endogenous semaphorins.

Many reports have described the essential roles of neuropilins in endothelial cell and neuronal function. The current study tells us that neuropilin-2 promotes colon carcinoma tumor progression in a mouse model. This is welcome new information. Let us hope that targeting neuropilin-2 can hit double punches against the cancer microenvironment and the cancer itself.

References