The role of neuropilins in cancer

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Abstract

Neuropilins are multifunctional non–tyrosine kinase receptors that bind to class 3 semaphorins and vascular endothelial growth factor. NRP-1 and NRP-2 were first identified for their key role in mediating axonal guidance in the developing nervous system through their interactions with class 3 semaphorins. Growing evidence supports a critical role for these receptors in tumor progression. Neuropilin expression is up-regulated in multiple tumor types, and correlates with tumor progression and prognosis in specific tumors. Neuropilins may indirectly mediate effects on tumor progression by affecting angiogenesis or directly through effects on tumor cells. This article reviews emerging evidence for the role of neuropilins in tumor biology. The therapeutic implications of these data are far-reaching and suggest that neuropilin-targeted interventions may be useful as a component of antineoplastic therapy. [Mol Cancer Ther 2006;5(5):1099–107]

Introduction

Neuropilin 1 (NRP-1) and neuropilin 2 (NRP-2) are multifunctional receptors that were first identified based on their critical role in the developing nervous system. In NRP-1 knockout mice, nerve trajectories are disorganized and limbs are not properly innervated (1). NRP-2 knockout mice exhibit defective development of cranial nerves and spinal sensory axons as well as disorganized or missing fiber tracts in the adult brain (2, 3). The neutralization of NRP-1 with blocking antibodies inhibits axonal repulsion, a necessary process in axonal guidance (4–7). The effects of neuropilins on the nervous system are mediated by binding to the semaphorins, a family of ligands that will be discussed in more detail below.

Subsequent investigations identified NRP-1 as a receptor for the vascular endothelial growth factor (VEGF)-A isoform, VEGF-165, on both endothelial cells and tumor cells (8, 9). This discovery led to investigations into the role of neuropilins in angiogenesis and tumor growth regulation. Data showing that neuropilin is expressed on tumor vasculature (10–13), is up-regulated in multiple tumor types (14–19), and that neuropilin expression correlates with tumor progression and patient prognosis in specific tumor types (10, 11, 14, 15, 20–22), suggests a role for neuropilins in tumor progression. This overview reviews neuropilins with a focus on the emerging evidence for their role in tumor biology.

Neuropilin Structure

Neuropilins are 120 to 130 kDa non–tyrosine kinase receptors (8, 9). Multiple NRP-1 and NRP-2 isoforms exist, including soluble forms (23, 24). The basic structure of neuropilins comprises five domains: three extracellular domains (a1a2, b1b2, and c), a transmembrane domain, and a short cytoplasmic domain (Fig. 1; ref. 25). The a1a2 domain is a CUB domain (named for its identification in complement components C1r and C1s, Uegf, and bmp1), a domain commonly found in developmentally regulated proteins and which generally contains four cysteine residues that make two disulfide bridges (26). The neuropilin CUB domain shares homology with complement components C1r and C1s (27). The first two extracellular domains of NRP-1 (i.e., a1a2 and b1b2) bind ligand (28). Additionally, the structure-function studies using neuropilin mutants containing deletions within the “a” and “b” domains show that the CUB domains (a1a2 and b1b2) are required for semaphorin binding (27). The third extracellular domain is critical for homodimerization or heterodimerization (28).

The cytoplasmic domain does not contain a kinase motif suggesting that it does not signal on its own. The cytoplasmic domains of NRP-1 and the NRP-2 splice variant, NRP-2a, have intracellular PSD-95/Dlg/ZO-1 (PDZ) binding motifs. NRP-1–interacting protein, a protein that has been shown to associate with NRP-1, contains a PDZ domain. PDZ domains are common protein-protein recognition modules in the nervous system. The PDZ domain on NRP-1–interacting protein binds to the PDZ binding motif on NRP-1 and NRP-2a and potentially represents a pathway by which neuropilins signal (29). More research is needed, however, to define the role, if any, of NRP-1–interacting protein in neuropilin signaling.

Neuropilins serve as receptors or coreceptors for multiple ligands. Known ligands include class 3 semaphorins, VEGF, heparin-binding proteins, fibroblast...
growth factor 2, and placental growth factor 2 (5, 24, 30–38). Of particular interest to the role of neuropilins in cancer biology are the ligands of the class 3 semaphorin and VEGF families.

Semaphorins are proteins initially recognized for their role in neuronal development. Semaphorins act as axonal chemorepellants that induce axon growth cone collapse, a process that guides neurons to their ultimate targets in the developing nervous system. Of eight existing classes, only class 3 semaphorins bind neuropilins. Among the class 3 semaphorins (i.e., SEMA3A-SEMA3F) those shown to date to bind neuropilins (i.e., SEMA3A, SEMA3B, and SEMA3F), exhibit antitumor properties. SEMA3A impedes tumor cell chemotaxis (30). SEMA3B and SEMA3F are candidate tumor suppressor genes and their expression is frequently lost in lung cancers (39, 40). SEMA3F has antiangiogenic properties that contribute to its antitumor effects (31). SEMA3A, SEMA3B, and SEMA3F compete with VEGF for neuropilin binding and seem to be mutually antagonistic (30, 32, 33, 39, 41). In addition, soluble NRP-1 may act as a VEGF antagonist (42).

The VEGF family is comprised of six proteins as described elsewhere (43–45). Three members of the VEGF family of ligands bind neuropilins: VEGF-A (hereafter referred to as VEGF), VEGF-B, and VEGF-C. The best characterized of these proteins is VEGF, which is a well-established proangiogenic factor (46) and a therapeutic target in cancer (43, 47).

**Neuropilin Signaling**

Initial investigations into neuropilin signaling suggested that neuropilins cannot transmit intracellular signals in isolation and that coreceptor complexes are required (i.e., adapter proteins that mediate downstream signaling; ref. 48). Indeed class 3 semaphorins signaling through NRP-1 seem to occur only in association with a coreceptor complex. This complex typically includes a plexin. Plexins are large transmembrane proteins that typically bind to semaphorins with the exception of most class 3 semaphorins (49). Plexins participate in class 3 semaphorin signaling by complexing with neuropilins to form a coreceptor. The plexins bind to NRP-1 via their Sema domain (28).

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**Figure 1.** Neuropilin structure. Neuropilin consists of three extracellular domains (a1a2, b1b2, and c), a transmembrane domain, and a short cytoplasmic domain. The cytoplasmic domains of NRP1 and NRP2A have PDZ binding motifs (adapted from ref. 25).

**Figure 2.** Class 3 semaphorin interaction with NRP-1. A, class 3 semaphorin signaling through NRP-1 occurs through a coreceptor complex consisting of a plexin and NRP-1. B, class 3 semaphorins bind to NRP-1 through both their SEMA and immunoglobulin domains and plexins bind to NRP-1 through their SEMA domains; however, the exact binding domains of the class 3 semaphorins with respect to this complex are not clearly understood (adapted from ref. 53).
responsible for the lesser potency of VEGF-121 compared with VEGF-165 (54). However, the exact mechanism by which the complex leads to differential potency between the two isoforms has yet to be fully elucidated (56). Other neuropilin/VEGFR complexes have been described: NRP-1 with VEGFR-1 (34, 57) and NRP-2 with VEGFR-1 (34). However, the functions of these complexes are less well characterized.

Several studies provide supporting evidence that VEGF can signal through neuropilins in the absence of VEGFR-1 or VEGFR-2 (8, 9, 58, 59). In order to determine if neuropilin signals in the absence of complexing with VEGFRs, Wang et al. developed a fusion protein combining the extracellular portion of the epidermal growth factor receptor with the transmembrane and intracellular portion of NRP-1, and transfected the receptor into human umbilical vein endothelial cells (58). The addition of epidermal growth factor to these cells that do not typically express epidermal growth factor receptors led to intracellular signaling, demonstrating that neuropilin can signal independent of VEGFR association. Another study using RNA interference–mediated silencing of NRP-1 showed that NRP-1 regulates endothelial cell adhesion to extracellular matrix proteins, a VEGF-mediated function, independently of VEGFR-2 (59). Finally, expression of neuropilins on tumor cells in the absence of VEGFR-1 and VEGFR-2 has been observed in several cancer types including pancreatic cancer (60), breast cancer (61, 62), and neuroblastoma (63). However, others have shown that VEGFRs are present on tumor cells, hence, in the absence of a more thorough evaluation of VEGFRs, the above studies require confirmation. The pathway by which VEGF signals through neuropilins in the absence of VEGFR-1 or VEGFR-2 has not been elucidated. However, it has been suggested that this signaling is mediated through NRP-1–interacting protein, a previously mentioned cytoplasmic protein that is thought to mediate signaling by interacting with GTPase activating proteins (29).

**Role of Neuropilin in Normal, Nonvascular Tissues**

**Expression**

The expression of NRP-1 has been shown in a variety of nonvascular cell types in nonpathologic conditions, suggesting a pleiotropic role for these receptors. Most notable for their role in nervous system development, NRP-1 is expressed in the developing nervous system of Xenopus tadpoles, mice, and chicks (64–66). In humans, neuropilin expression has been detected in osteoblasts (67, 68), neuroendocrine cells of the gastrointestinal tract (69), dendritic cells (70–72), T cells (70), bone marrow fibroblasts and adipocytes (73, 74), renal glomerular mesangial cells (75), and glomerular epithelial cells (76). Knowledge of the conditions governing regulation of neuropilin expression in normal, nonvascular tissues is limited, but has been more widely explored in tumors (see below).

**Function**

Numerous studies employing animal models deficient in NRP-1 or NRP-2 have shown the key role of neuropilins in the developing nervous system. NRP-1- and NRP-2-deficient animals exhibit abnormal neural trajectories and inappropriate projection of a number of different classes of neurons (1–3, 77–80). These effects are thought to be mediated by defects in axonal repulsion (5, 81, 82).

Despite the widespread expression of NRP-1 in normal nonvascular tissues, the functional role played by neuropilin in systems other than the nervous system is not well defined. One report suggests a role for neuropilin in dendritic cell–T cell interactions because dendritic cell–induced proliferation of resting T cells is inhibited by anti-NRP-1 antibodies (70).

**Role of Neuropilin in Angiogenesis**

**Expression**

Neuropilins are widely expressed in normal mature and developing vasculature both on vascular smooth muscle cells (11, 83) and endothelial cells (50). Their presence has also been detected in tumor vascular endothelium (10–13), an important finding with regard to their role in promoting pathologic angiogenesis in malignancy.

Factors governing the regulation of neuropilin expression on endothelial cells are better defined than those in nonvascular tissues. Focal ischemia induces NRP-1 expression on endothelial cells of cerebral blood vessels (84). Several growth factors also regulate neuropilin expression. The expression of NRP-1, but not NRP-2, on endothelial cells is increased by VEGF, mediated through VEGFR-2 (85). Experiments with tumor necrosis factor regulation have shown conflicting results of both increased and
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...tions suggest that neuropilins mediate proangiogenic effects by binding to VEGF, and antiangiogenic effects by interaction with the class 3 semaphorins, where it is hypothesized that they compete for VEGF binding. Other studies suggest that SEMA3 proteins potentially regulate integrin-mediated adhesion and migration (93). The disruption of endogenous SEMA3 function in endothelial cells stimulates integrin adhesion and migration to extracellular matrix components. It is believed that SEMA3 family members control vascular plasticity via the regulation of integrins (93).

The direct effects of VEGF on endothelial cells are well known and the role of NRP-1 in mediating these effects is being elucidated. Signaling through NRP-1, VEGF stimulates endothelial cell migration and adhesion, important properties for the early stages of angiogenesis. The addition of an anti-NRP-1 antibody suppressed the mitogenic effects of VEGF-165 on bovine retinal endothelial cells (85). In an in vitro model, the VEGF-dependent differentiation of human bone marrow AC133+ cells into vascular precursors, and subsequent proliferation of these cells, required the activation of a VEGFR-2/NRP-1-dependent signaling pathway (94). Finally, VEGF also promotes the synthesis and release of prostacyclin (prostaglandin I2), a mediator involved in angiogenesis, as well as being a regulator of vascular tone. These effects are thought to be mediated via NRP-1 binding (95). The angiogenic effects mediated through VEGF binding to NRP-2 are less well characterized and seem to be regulated differently from NRP-1. For example, VEGF selectively up-regulates NRP-1 but not NRP-2 on endothelial cells (85).

At least two members of the class 3 semaphorin family exhibit antiangiogenic activity mediated through neuropilins. Utilizing porcine aortic endothelial cells that differentially express NRP-1, VEGFR-2, or both, it was determined that SEMA3A inhibited endothelial cell motility via NRP-1 (41). Other studies have shown the antiangiogenic activity of SEMA3F. In these studies, SEMA3F inhibited VEGF- and basic fibroblast growth factor–induced endothelial cell proliferation and survival, as well as VEGF- and basic fibroblast growth factor–induced angiogenesis in an in vitro alginate capsule system and Matrigel plug system, respectively. The lack of basic fibroblast growth factor binding to neuropilins, combined with the fact that SEMA3F did not inhibit binding of basic fibroblast growth factor to its receptor, suggests that SEMA3F is acting through NRP-2 (96). It is thought that these effects were mediated through NRP-2 because NRP-1 has lower affinity for SEMA3F and, unlike NRP-2, does not transduce a signal when it binds to SEMA3F (97, 98); however, determining the mediator of these effects requires further study. Further experiments showed that SEMA3F inhibits tumor angiogenesis in vivo. Highly metastatic melanoma cells (A375SM) were transfected with SEMA3F and injected subdermally into mice (31). These SEMA3F-expressing tumors had reduced vascularization compared with non–SEMA3F-expressing control tumors. To further evaluate the mechanisms governing the inhibition of angiogenesis in this system, the SEMA3F-expressing melanoma cells were cocultured with porcine aortic endothelial cells transfected with either NRP-1 or NRP-2. SEMA3F exhibited repulsive activity only in NRP-2-expressing endothelial cells. Using RNA interference, NRP-2 was silenced and the repulsive effects of SEMA3F were inhibited. Corroborative experiments were conducted that used lymphatic endothelial cells, which express NRP-2 but not NRP-1. These experiments confirmed the role of NRP-2 in SEMA3F-induced endothelial cell repulsion.

Taken together, these experiments suggest that, acting through NRP-1 or NRP-2, VEGF promotes angiogenesis, whereas SEMA3A and SEMA3F inhibit angiogenesis. Semaphorins theoretically compete with VEGF for binding to neuropilin, a property not only important for endothelial cell function, but also important for tumor cell function, as discussed below.

Role of Neuropilins on Tumor Cells

Expression

Both NRP-1 and NRP-2 are expressed on several types of tumor cells (Table 1); in many cancers, expression of one or both have been correlated with tumor progression and/or poorer prognosis. In human tumors, the expression of NRP-1 correlates with tumor growth and invasiveness in prostate cancer (20), colorectal cancer (14, 21), lung cancer (15), breast cancer (11), and astrocytomas (10, 22). Interestingly, in animal models, tumor cell expression of NRP-1, without concomitant increased endothelial NRP-1 expression, has been shown to promote tumor angiogenesis (21, 99). This finding may explain, in part, the observation in our laboratory that the expression of NRP-1 correlates with the extent of angiogenesis in a xenograft model of colorectal cancer (21). However, interestingly, in pancreatic...
cancer cells, NRP-1 overexpression led to a decrease in tumor growth (99), and in subsequent experiments, knockdown of endogenous NRP-1 expression with RNA interference led to an increase in tumor growth (100). This is an isolated finding and the mechanism for this is currently under investigation in my laboratory. However, this highlights the complexity of the neuropilin system, and thus, the need for more investigation.

The expression of NRP-2 is variably correlated with tumor progression and prognosis in human cancer. In bladder cancer, NRP-2 expression correlates with advanced tumor stage and grade (101). In non–small cell lung cancers, the expression of both NRP-1 and NRP-2 are increased relative to nonneoplastic tissues (16). Interestingly, in patients in which the tumor cells coexpressed NRP-1 and NRP-2 mRNA, the prognosis was worse. Also of interest is a study in gastrointestinal carcinoid tumors in which loss of NRP-2 expression correlated with tumor progression (69). Because NRP-2 is a receptor for SEMA3F, it is possible that the loss of SEMA3F activity contributes to malignant progression in these tumors, however, further study is clearly required to establish such a link.

A number of environmental mediators have been found that regulate neuropilin expression in tumor cells. Experiments with hypoxia, a key regulator of VEGF expression, have shown conflicting results regarding neuropilin expression in different tumor systems, and this effect may be cell type–specific. Hypoxia leads to increased expression of NRP-1 in neuroblastoma cells but decreased expression in astrocytoma cells (22, 102). Insulin-like growth factor-1 increased NRP-1 expression on colon tumor cells (18, 21), but tumor necrosis factor, a cytokine that has been shown to increase neuropilin in other tumor systems, failed to increase NRP-1 expression in these same cells. Activation of epidermal growth factor receptors has also been shown to up-regulate NRP-1 expression in several tumor systems (18, 21, 22, 103). Studies from our laboratory have shown that epidermal growth factor induces NRP-1 expression in human colon and pancreatic adenocarcinoma cell lines, which seems to be mediated through the phosphatidylinositol-3 kinase/Akt and P38 mitogen-activated protein kinase/extracellular signal-regulated kinase 1/2 signaling pathways (18, 21).

**Function**

A number of experiments have sought to elucidate the functional role of neuropilins on tumor cells. Results from *in vitro* studies have been published in breast, pancreatic, colon, and prostate cancers and melanoma.

**Breast cancer.** Several studies in human breast cancer cell lines have implicated NRP-1 in various aspects of cell growth, survival, migration, and metastasis. In breast cancer models, the NRP-1-expressing human breast cancer cell line, MDA-MB-231, which lacks VEGFR-2, was exposed to two VEGF isoforms (VEGF-165, which binds NRP-1, and VEGF-121, which does not; ref. 61). VEGF-165 prevented apoptosis, whereas VEGF-121 did not, suggesting that VEGF provides a survival signal for these cells and that the signal is transduced through NRP-1. Further experiments with this cell line showed that NRP-1 expression also protected cells from hypoxia-induced apoptosis (61). Additional evidence for the role of NRP-1 in VEGF-induced protection from apoptosis comes from a study that employed an anti–NRP-1-binding peptide that, when added to MDA-MB-231 cells, induced apoptosis (62).

Semaphorin-3B may antagonize the antiapoptotic effects of VEGF in breast cancer cells. In MDA-MB-231 cells, SEMA3B inhibited tumor cell growth and induced apoptosis (32). This effect was reversed by VEGF-165 but not VEGF-121, an observation that, in conjunction with other confirmatory experiments, suggests that this activity is mediated through NRP-1.

In addition to the role of neuropilin in mediating proapoptotic and antiapoptotic signals in breast cancer cells, neuropilin may also mediate breast cancer cell migration and metastasis. Cell spreading and membrane ruffling are inhibited by SEMA3F in the breast cancer cell line, MCF7, which expresses NRP-1 but not NRP-2, and in the breast cancer cell line, C100, which expresses NRP-2 and lower levels of NRP-1 (33). In contrast, VEGF promotes membrane ruffling in these cell lines. In these studies, VEGF activity was blocked by SEMA3F, suggesting that SEMA3F competes with VEGF for binding to neuropilins. Use of anti-NRP-1 and anti-NRP-2 antibodies confirmed that these effects were due to SEMA3F interaction with NRP-1 in the MCF7 cells and NRP-2 in the C100 cells. In subsequent experiments in C100 cells, it was shown that SEMA3F inhibited cell migration (104). This effect was mediated by NRP-2 as it was blocked by anti-NRP-2 antibodies but not by anti-NRP-1 antibodies. SEMA3F inhibited cell-cell adhesion in MCF7 cells through a loss of membrane E-cadherin expression (104). This effect was mediated through NRP-1. Because loss of cell-cell contact in a tumor mass is one step in the process of metastasis (104), these experiments suggest that SEMA3F would promote metastasis. However, in this study, loss of E-cadherin was not accompanied by an increase in

### Table 1. Neuropilin expression on tumor cells

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>NRP-1</th>
<th>NRP-2</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder cancer</td>
<td>×</td>
<td>×</td>
<td>(101)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>×</td>
<td></td>
<td>(61, 62)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>×</td>
<td></td>
<td>(14, 21)</td>
</tr>
<tr>
<td>Esophageal cancer</td>
<td>×</td>
<td></td>
<td>(14)</td>
</tr>
<tr>
<td>Gall bladder cancer</td>
<td>×</td>
<td></td>
<td>(14)</td>
</tr>
<tr>
<td>Glioma</td>
<td></td>
<td></td>
<td>(10, 22)</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>×</td>
<td>×</td>
<td>(63)</td>
</tr>
<tr>
<td>Non–small cell lung cancer</td>
<td>×</td>
<td>×</td>
<td>(15)</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>×</td>
<td>×</td>
<td>(14, 17, 60, 69)</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>×</td>
<td>×</td>
<td>(20, 111)</td>
</tr>
<tr>
<td>Small cell lung cancer</td>
<td>×</td>
<td>×</td>
<td>(15)</td>
</tr>
</tbody>
</table>

NOTE: Not all tumor cell lines have been examined for both NRP-1 and NRP-2.
N-cadherin expression, a process (i.e., cadherin switching) common in tumor progression (105). In addition, loss of E-cadherin in these experiments was not associated with increased cell motility or migration (104).

Other class 3 semaphorins also inhibit breast cancer cell migration. In vitro studies showed that SEMA3A inhibits the migration of breast cancer cell lines through an NRP-1/plexin-1A pathway. This activity was antagonized by VEGF (30). Consistent with this, studies using in vitro Matrigel assays showed that VEGF promotes breast cancer cell migration (106). In this system, invasion was inhibited by VEGF antisense constructs and restored with recombinant VEGF. This effect was mediated by NRP-1 (106). Thus, it seems that NRP-1 can mediate both promigratory and antimigratory activities depending on the particular ligand.

Further support for the competing promigratory and antimigratory effects of VEGF and SEMA3A on breast cancer cells is derived from experiments in which the protein levels of breast cancer cell lines were quantified and correlated with the chemotactic activity of the cells (30). Cells with the highest SEMA3A/VEGF protein ratio exhibited the lowest chemotactic rate. In contrast, those that had the lowest ratio of SEMA3A/VEGF protein exhibited the highest chemotactic rates. Furthermore, when the SEMA3A/VEGF protein ratio was increased by decreasing VEGF levels with VEGF antisense, the chemotactic activities of the cells decreased. The chemotactic rate was restored when SEMA3A protein was reduced by transfection with a SEMA3A RNA interference–expressing retrovirus.

Taken together, these studies suggest that VEGF and SEMA3A and SEMA3F play key and potentially antagonistic roles in breast tumor cell migration. Further studies are necessary to dissect the complex interplay between these factors and to understand the signaling pathways mediating these effects.

**Pancreatic cancer.** NRP-1 seems to play diverse, and at times, unclear roles in pancreatic carcinoma cell proliferation, apoptosis, and chemoresistance. In a study with the pancreatic carcinoma cell line Panc-1, VEGF induced cell proliferation through NRP-1 in the absence of detectable VEGFRs (60). However, it is difficult to draw conclusions about the role of NRP-1 in this system because we have recently shown the presence of functional VEGFR-1 on Panc-1 cells (107). In the human pancreatic cell line FG, NRP-1 overexpression was associated with constitutive activation of mitogen-activated protein kinase signaling pathways, extracellular signal-regulated kinase 1/2, and c-Jun-NH2-kinase (108).

Using conditioned medium from the overexpressing cells, it was determined that an unidentified secreted factor is likely responsible for the induction of signaling, although the authors’ studies did not suggest that this was mediated through VEGF or semaphorins.

The overexpression of NRP-1 also inhibited detachment-induced apoptosis (108). Microarray analysis suggested that NRP-1 may mediate signals that lead to induction of antiapoptotic genes (108). Finally, NRP-1 overexpression resulted in in vitro chemoresistance of human pancreatic cancer cells to gemcitabine and 5-fluorouracil; reduction of NRP-1 expression levels by siRNA in Panc-1 cells increased chemosensitivity to gemcitabine (108).

**Colon cancer.** In preclinical studies, NRP-1 was found to play a role in the growth and metastasis of colon cancer. The human colon adenocarcinoma cell line, KM12SM/LM2, transfected with NRP-1 and injected into nude mice, produced tumors of greater mass, volume, and number of vessels compared with the same cell line transfected with a control vector (21).

**Prostate cancer.** NRP-1 may play a role in promoting tumor angiogenesis and tumor growth in prostate cancer. When rat prostate carcinoma cells were engineered to express high levels of NRP-1 via a tetracycline-inducible promoter, in vivo tumor growth increased 2.5-fold to 7-fold and the tumors were markedly more vascular (i.e., 3-fold to 4-fold greater numbers of blood vessels; ref. 99).

In addition, tumors induced to express NRP-1 showed very few apoptotic tumor or endothelial cells compared with controls. These effects may have been mediated by VEGF binding as sections from NRP-1-expressing tumors showed markedly increased VEGF expression relative to non–NRP-1-expressing tumors.

**Melanoma.** Semaphorins may reduce tumor growth and metastasis. In addition to the antiangiogenic effects of SEMA3F mediated through NRP-2 in the experimental metastatic melanoma study described previously, direct antitumor effects were observed (31). When A375SM cells, transfected with SEMA3F, were injected s.c. into mice, the resulting tumors had areas of apoptosis and nondividing cells in addition to reduced numbers of vessels. Importantly, these tumors did not metastasize, either spontaneously or experimentally when tumor cells were injected into the tail vein.

**Therapeutic Implications**

The growing body of evidence supporting a substantial and independent role for neuropilins in cancer opens the possibility for the use of antitumor therapies that target these receptors. Several potential, yet unproven therapeutic options for targeting neuropilins include (a) blockade of signaling via inhibiting ligand binding of stimulatory factors (i.e., bevacizumab, a monoclonal antibody to VEGF), (b) blockade of signaling by increasing ligand binding of inhibitory factors (e.g., SEMA3A), or (c) direct blockade of neuropilin activity with antibodies or RNA interference.

Reduced neuropilin activity has also been shown with a few agents with diverse mechanisms of action. In human umbilical cord endothelial cells, the histone deacetylase inhibitors, trichostatin-A and suberoylanilide hydroxamic acid, inhibited VEGF-induced expression of NRP-1 (109).

Depletion of NRP-1 has also been shown to be induced by thalidomide in a zebrafish model (110).

Direct targeting of neuropilin has been achieved experimentally using a peptide that binds NRP-1 (62).
Exposing murine and human breast carcinoma cells to the peptide resulted in apoptosis via blockade of NRP-1, which inhibited VEGF binding and its antiapoptotic activity.

Summary
Neuropilins are multifunctional receptors that mediate critical functions in the nervous system, vascular system, and tumor cells. Neuropilins serve as receptors for the class 3 semaphorin and VEGF family members. Neuropilins are expressed in the tumor vasculature and on tumor cells, and their expression has been correlated with tumor angiogenesis and tumor progression. In general, functions mediated through neuropilins in tumor cells and tumor-associated endothelial cells include VEGF-induced proangiogenic effects, including the promotion of endothelial cell motility and adhesion, and semaphorin-induced antiangiogenic effects; semaphorins seem to serve as antagonists to VEGF and have the opposite effects. Understanding the interaction of VEGF, VEGFRs, semaphorins, and neuropilins should provide guidance for the rational development of novel anticancer strategies and the optimal use of approved agents.

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