Neuropilins in Tumor Biology

Anil Bagri,1 Marc Tessier-Lavigne,2 and Ryan J. Watts3

Abstract

The neuropilin receptors were first discovered as regulators of nervous system development, acting as semaphorin coreceptors with plexins. Subsequently, the neuropilins were identified as receptors for vascular endothelial growth factor. Since those seminal discoveries, additional ligands that bind neuropilins have been described, and many studies have implicated neuropilins in playing key roles in tumor biology. Recent evidence has shown that manipulating neuropilin function can regulate tumor growth and metastasis through effects on vascular biology in the case of neuropilin-1 and lymphatic biology in the case of neuropilin-2. A direct role for neuropilins within tumor cells has also been postulated. As data continue to accumulate pointing to a role for neuropilins in cancer, the promise for targeting this pathway is beginning to unfold.

Background

The neuropilin receptors (Nrp1 and Nrp2) are single-pass transmembrane glycoproteins with three structural motifs: two CUB homology domains (a1, a2), two coagulation factor V/VIII homology domains (b1, b2), and a MAM domain (c; see Fig. 1). In addition to having similar motifs, Nrp1 and Nrp2 share 44% amino acid sequence identity. They also have multiple ligands and coreceptors (Fig. 1). The most studied ligands include semaphorins (1, 2), which interact principally with the a1-a2 domains, and vascular endothelial growth factor (VEGF) family members (3), which bind the b1-b2 domains (for an extensive review on ligand interactions, see ref. 4).

The promiscuous binding of the Nrp receptors with multiple ligand families has resulted in the discovery of varied biological functions, particularly in neural and vascular development. A common thread has emerged from these studies, in that Nrp1 seems to have as a principal function to regulate cell motility, including neuronal growth cone collapse mediated by semaphorins, and vascular and lymphatic endothelial sprouting triggered by VEGFs (Fig. 1). Nrp1 have become a focus of tumor biology studies, both because of their involvement in vascular and lymphatic development, and because of potential roles within tumor cells.

Nrp1 in Tumor Biology

Vascular targeting. Many studies have explored the role of Nrp1 in vascular biology. Klagsbrun and colleagues first proposed that Nrp1 function as VEGF receptors to regulate vascular development (3). This theory was supported by genetic analysis. In particular, loss of Nrp1 function, either systemically (5–8) or selectively in blood vessels (9), resulted in vascular sprouting and remodeling defects. These findings, together with advances in blocking blood vessel formation and survival by inhibiting VEGF function in mouse tumor models and in human cancers (10), led researchers to postulate that blocking Nrp1 function may affect tumor growth by targeting the vasculature.

A first wave of experiments aimed at determining whether Nrp1 is a useful therapeutic target largely focused on using Nrp1 ectodomains or soluble Nrp1 (sNrp1) as blocking reagents. Expressing sNrp1 in tumor cells (rat AT2.1 or AT3.1 prostate carcinoma cells) resulted in reduced tumor growth and an increase in tumor cell apoptosis (11). In a follow-up to this study, Klagsbrun and colleagues (12) used the same cell lines to overexpress Nrp1; an increase in tumor vascular density in tumors expressing high levels of Nrp1 was observed. These results led the investigators to propose a model in which Nrp1 can act in trans to enhance VEGF signaling on endothelial cells, subsequently leading to increased tumor vascular density.

Two additional studies used Nrp1 ectodomains to attempt to block Nrp1 function. Kuo et al. (13) administered adenovirus systemically to express sNrp1 and showed no change in tumor growth of either Lewis lung carcinoma or T241 fibrocarcoma. In contrast, using a microencapsulation technique to express sNrp1 adjacent to s.c. M1 chloroma tumor, it was shown that sNrp1 expression reduced both tumor volume and vascular density (14). In this study, it was also shown that systemic administration of sNrp1 via adenovirus prolonged survival in a leukemic mouse model. More recently, it has been reported that a small peptide-mimetic of VEGF selectively blocks VEGF binding to Nrp1 and inhibits angiogenesis and reduces tumor growth (15).

These studies laid the groundwork for defining a role for Nrp1 in tumor growth by vascular mechanisms. It was difficult, however, from these initial studies to separate an...
antiangiogenic effect from an antitumor effect. To further address this issue, more targeted tools for blocking Nrp1 function were generated (16, 17). Specifically, phage-derived function-blocking antibodies directed to either the a domains (anti-NRP1A, blocking Sema3A binding) or the b domains of Nrp1 (anti-NRP1 B, blocking VEGF binding) were engineered. Focusing on anti-NRP1 B (YW107.4.87), it was shown that systemic administration of this antibody slowed tumor growth in both a lung carcinoma model (Calu6), which expresses relatively high levels of Nrp1 on tumor cells. Anti-NRP1 B also significantly slowed tumor growth in a colon carcinoma model (HM7), which does not express Nrp1. In the HM7 model, a reduction in vascular density as measured by histology and micro-CT was also observed.4

These data provided evidence for a vascular-specific role for Nrp1 in tumor-mediated angiogenesis, although they do not exclude the possibility of additional tumor-specific function for Nrp1 in some cases (see further). The data also raised the question of whether anti-NRP1 effects would combine with anti-VEGF therapy. Studies to address this were done in mouse models of lung carcinoma, where anti-NRP1 and anti-VEGF were coadministered (17). The results showed an additive effect in reducing tumor growth and vascular density when anti-NRP1 was combined with anti-VEGF. Based on detailed cell biological analysis, including studies of the role of Nrp1 in developmental vascular biology in the retina, a model was proposed in which anti-NRP1 through its anti-vascular remodeling effects keeps blood vessels in a VEGF-dependent state, so that when it is combined with anti-VEGF, additional vascular regression is observed beyond that seen with anti-VEGF treatment alone.

**Lymphatic targeting.** The importance of lymphatic vasculature to metastasis is widely accepted, as evidenced by the clinical practice of regional lymph node analysis for micrometastatic disease as a key prognostic indicator. Recent studies suggest that targeting lymphatics and lymphangiogenesis may be a useful therapeutic strategy to restrict cancer metastasis, most commonly, by inhibition of VEGF-C signaling with VEGFR3 ectodomain proteins or anti-VEGFR3 antibodies (18, 19).

Nrp2, like Nrp1, can bind to members of the VEGF family of growth factors, including VEGF-A, VEGF-C, and VEGF-D, and there is mounting evidence supporting the role of Nrp2 in VEGF-C/D signaling through VEGF receptors (primarily VEGFR3; refs. 20, 21). Furthermore, whereas Nrp2 knockout
mice do not exhibit defects in blood vessels, they display abnormal lymphatic development (22), including an abnormal patterning and marked reduction in small lymphatic vessel and capillaries, supporting a role for Nrp2 in VEGF-C mediated VEGFR3 signaling and developmental lymphangiogenesis.

The importance of Nrp2 in modulating lymphatic metastasis has also been recently shown using a phage-derived function-blocking antibody directed against the b1b2 or VEGF-binding domains of Nrp2 (Anti-NRP2β, ref. 23). Treatment of mouse tumor models with this antibody results in an inhibition of tumor lymphangiogenesis and a reduction in functional lymphatics associated with tumors. As a consequence, there is a reduction in metastasis to sentinel lymph nodes and distant organs. Anti-NRP2β does not affect established lymphatics in normal and tumor-bearing adult mice, which is important when considering potential therapeutic utility. Interestingly, this study also provides evidence that the function of Nrp2 extends beyond its previously assigned role as an enhancer of VEGF receptor activation.

Nrp2 has also been implicated in modulating metastasis via additional mechanisms. In colorectal carcinoma cells, inhibition of Nrp2 by shRNA results in marked reduction of anchorage independent growth, motility, invasiveness, and survival under hypoxic conditions of tumor cells (24). These effects are thought to contribute to a 60% to 80% reduction in hepatic metastasis (in comparison with control shRNA-treated tumor cells) seen with splenic implantation of these Nrp2 knockdown tumor cells. In contrast, sema3F, for which Nrp2 is the high-affinity receptor, has been shown to inhibit the invasion of melanoma tumor cells in vitro. This reduced invasive behavior and the formation of a well-encapsulated tumor resulted in reduced metastasis of sema3F overexpressing melanoma tumor cells (25). Thus, inhibition of Nrp2 may have differential effects on modulating metastasis, depending on the mechanism through which it acts. Regardless, these data implicate Nrp2 as an important modulator of metastasis.

Direct role for the neuropilins in tumor cell function. The Nrps are expressed in a wide variety of human tumor cell lines including those derived from carcinomas of the prostate, kidney, bladder, stomach, colon, pancreas, breast, ovary, and lung (reviewed in refs. 26 and 27). They are also expressed in a number of glioblastoma, neuroblastoma, osteosarcoma, leukemia, and melanoma cell lines. The expression of shRNA or Nrp2 has also been detected by immunostaining in the tumor cells of patient tumor samples or biopsies from many of these tumor types. Additionally, increased expression of one or both Nrps correlates with tumor aggressiveness, disease stage, or poor prognosis in tumors from prostate, lung (non–small cell lung cancer), ovary, colon, and other gastrointestinal tract organs, and also gliomas and osteosarcomas (reviewed by refs. 26, 27).

Nrp1 and Nrp2 have also been implicated in mediating proliferation, survival, and migration of tumor cells. Overexpression of Nrp1 in Dunning rat prostate carcinoma AT2.1 cells increased tumor cell migration and also resulted in increased tumor growth and reduced tumor cell apoptosis in vivo (12). Increased microvascular density was also observed in these tumors, however, and the relative importance of the indirect vascular effects as opposed to direct tumor cell effects is unclear. In separate experiments, Nrp1 overexpression in tumor cells resulted in reduced hypoxia-induced apoptosis (28) and inhibition of Nrp1 with a blocking peptide resulted in increased apoptosis (29) of breast adenocarcinoma cells. Nrp1 inhibition with an antibody reduced invasion of breast tumor cells (30).

Although these studies have indicated a protumorigenic role of Nrps, evaluation of semaphorin effects on tumor cells suggests that Nrps may play a more complex role in some tumor types. Sema3A, a Nrp1 ligand, inhibited the migration and spreading of breast cancer cells and the invasiveness of prostate cancer cells in vitro, but the in vivo importance of this observation remains uncertain (30, 31). Furthermore, there have also been contradictory reports suggesting that Sema3A contributes to the progression of pancreatic cancer and colon cancer (32, 33). A stronger case has been made for Nrp2 binding the semaphorin Sema3F. Genetic studies originally implicated Sema3F as a tumor suppressor of small cell lung carcinoma tumors (34) and expression of recombinant Sema3F in small cell lung carcinoma cells inhibited colony formation in soft agar, indicating that Sema3F directly affects the behavior of these cells (35). Sema3F expression in breast carcinoma cells inhibited their adhesion and spreading, potentially mediated by loss of E-cadherin (36, 37). Additionally, Sema3B, another Nrp ligand, was shown to inhibit anchorage-independent growth and induce apoptosis of lung and ovarian carcinoma cells (38, 39).

These data are consistent with the idea that Nrps expressed by tumor cells may have a functional role and directly contribute to disease progression. It is important to note, however, that in addition to VEGF and semaphorin family members, additional ligands have been identified that can bind and may act through Nrps (4). Further studies are necessary to dissect the complex interplay between all of these factors in modulating tumor biology.

Clinical-Translational Advances

Improved understanding of the role of Nrps in modulating semaphorin- and VEGF-regulated biologies has provided opportunities for therapeutically targeting Nrps in tumor biology. Preclinically, a number of strategies have been used to inhibit Nrp function, including knockdown strategies with siRNAs, small peptide inhibitors, and function blocking antibodies. The latter approach, in particular, is attractive as it allows specific targeting of either VEGF- or semaphorin-mediated Nrp function (17, 23). This is particularly attractive as VEGF family ligands and semaphorins may have antagonistic effects on tumor progression in some tumors. Thus, identification of the role that Nrp plays in a given patient’s tumor, through the development of diagnostic tests, may allow selection of which axis (if any) is the one that is most important to block to maximize patient benefit.

Although the direct role of Nrps on tumor cells is complex and remains controversial, the role of Nrps, particularly Nrp1, in tumor angiogenesis provides a clear opportunity for realizing benefit by inhibiting Nrp function. As discussed above, inhibition of Nrp1 provides maximal benefit in combination with VEGF inhibition (17). Currently, there are a number of agents in the clinic and in clinical trials that inhibit the VEGF axis, including bevacizumab (Avastin; Genentech, Inc.),
a humanized monoclonal anti-VEGF antibody approved for clinical use in patients. It is attractive to speculate that combination of anti-NRP1 with Avastin or other VEGF-inhibiting agents could provide improved antiangiogenic effects in patients as well.

Additionally, whereas Nrp1-/- mice die between E10 and E13.5, and Nrp2-/- mice are viable and exhibit no blood vessel defects, inactivation of both Nrp genes results in much more severe cardiovascular defects resulting in lethality at E8 (6). This suggests that both Nrpps may play a role in angiogenesis, or that compensation for Nrp1 by Nrp2 may be possible. Although it remains to be tested, it is also possible that dual inhibition of Nrp1 and Nrp2 in tumors may provide additional benefit, compared with inhibition by Nrp1 alone, suggesting additional therapeutic combinations.

At present, no antilymphangiogenic strategies have been directly evaluated in the clinic. It has been proposed that such treatment during neoadjuvant therapy may restrict metastasis during a period designed to facilitate surgery, or for patients who are undergoing adjuvant therapy because of high risk of disease recurrence. Additionally, this strategy may slow further development of metastases in patients who are on palliative therapy due to nonresectable lesions. At present, however, it is unclear how effective these approaches may be.

With over a decade of intense research on the Nrps and their roles in various biological mechanisms, there is building evidence that these are attractive cancer targets. Nevertheless, as therapies move forward into clinical trials targeting the Nrp pathway, it is clear that much remains to be understood about the mechanisms underlying these complex multiligand receptors.

Disclosure of Potential Conflicts of Interest
The authors are employees of Genentech, Inc.

References

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