Journal Club

The good, the bad and the ugly
A neuropilin-2 story from normal to tumor-associated lymphangiogenesis

Lise Roth
INSERM U575 Centre de Neurochimie; Strasbourg, France

Abbreviations: BrDU, 5-bromo-2-deoxyuridine; LEC, lymphatic endothelial cell; LYVE-1, lymphatic vascular endothelial hyaluronan receptor-1; NRP, neuropilin; PECAM-1, platelet-endothelial cell adhesion molecule-1; SLN, sentinel lymph node; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; VEGFR3ECD, VEGFR-3 extracellular domain

Key words: VEGF-C, NRP2, VEGFR-3, tumoral lymphangiogenesis, metastasis

VEGF-C is a crucial player in lymphangiogenesis. Besides VEGF-R2 and VEGF-R3, it also binds NRP2. NRP2 enhances VEGF-C/VEGFR-3 effects in developmental lymphangiogenesis, but its role in adult and tumoral lymphangiogenesis is not known. In their study, Bagri and colleagues demonstrate that blocking NRP2 results in a decrease of metastasis formation, a phenomenon relying on tumoral lymphangiogenesis. Thus, they identified NRP2 as an attractive new target for modulating metastasis.

Besides developmental and adult lymphangiogenesis, formation of new lymphatic vessels also occurs during tumor growth. This abnormal lymphangiogenesis enables tumor cells to escape from the solid tumor and to invade the body through the newly formed lymphatics. The resulting metastases are responsible for most cancer-associated deaths. Interestingly, inhibition of VEGF-C/VEGFR-3 axis has been shown to block metastasis formation in preclinical models. However, treatment with VEGFR3ECD may result in compromise of the normal lymphatic system leading to complications such as lymphedema. VEGF-C belongs to the VEGF family and is particularly involved in lymphangiogenesis. VEGF-C binds VEGFR-2 but mostly associates with high affinity with the third tyrosine kinase VEGF receptor, VEGFR-3. Recently, it has been proved that VEGF-C also binds a receptor from the neuropilin family, NRP2. NRP2 associates with VEGF-C and binding of VEGF-C to NRP2 enhances VEGF-C/VEGFR-3-induced biological effects. NRP2 thus modulates developmental lymphangiogenesis, but its significance in adult or tumoral lymphangiogenesis remained unknown until a recent study by Bagri and colleagues who analyzed NRP2 function in tumor cell metastasis.

NRP2 Mediates Selective VEGF-C-induced Effects

The authors generated a specific and high-affinity monoclonal antibody to the VEGF-binding region of NRP2, anti-NRP2B. NRP2 involvement in VEGF-C-induced lymphatic endothelial cell migration and proliferation was first evaluated in vitro. Using a transwell system, the authors determined that anti-NRP2B specifically inhibits VEGF-C-induced migration, but to a lesser extent than blocking VEGFR-3 using VEGFR3ECD. Interestingly, unlike VEGFR3ECD, which totally inhibited VEGF-C-induced LEC proliferation, anti-NRP2B induced no decrease of BrDU incorporation by LEC. Thus NRP2 selectively mediates in vitro VEGF-C cellular effects. Moreover, this functional selectivity was confirmed in vivo because anti-NRP2B blocked VEGF-C-induced corneal lymphangiogenesis equivalently to VEGFR3ECD but had no effect on VEGF-C-induced vascular permeability, as revealed by a mouse skin vessel permeability assay. The authors then aimed to explain the mechanism by which anti-NRP2B is able to inhibit selective VEGF-C actions, both in vitro and in vivo. After having ruled out a general adhesion/migration inhibition by anti-NRP2B, or anti-NRP2B-induced internalization, they reasoned that the selective anti-NRP2B inhibitory activity could be due to differential requirements of VEGFR activation for migration and proliferation. Anti-NRP2B could then modulate VEGF-induced intracellular signalling, which would selectively affect VEGF-C-induced migration. However, anti-NRP2B only slightly inhibited VEGF-3 phosphorylation, and did not significantly reduce VEGF-3 effector activation. Furthermore, an amount of VEGF-C corresponding to VEGFR phosphorylation inhibition (150–175 ng/ml) had no effect on LEC migration. This clearly demonstrates that NRP2 function is not limited to an enhancement of VEGFR activation or downstream signalling. Anti-NRP2B could then modulate VEGF-induced intracellular signalling, which would selectively affect VEGF-C-induced migration. However, anti-NRP2B only slightly inhibited VEGF-3 phosphorylation, and did not significantly reduce VEGF-3 effector activation. Furthermore, an amount of VEGF-C corresponding to VEGFR phosphorylation inhibition (150–175 ng/ml) had no effect on LEC migration. This clearly demonstrates that NRP2 function is not limited to an enhancement of VEGFR activation or downstream signalling. As NRP2 can form a complex with VEGFR-2 or VEGFR-3, the authors next speculated that anti-NRP2B could affect the formation of this receptor complex. Indeed, the NRP2/VEGFR-3 interaction was strongly inhibited in presence of anti-NRP2B, suggesting that the NRP2/VEGFR-3 complex is important for specific VEGF-C-mediated functions.

Correspondence to: Lise Roth; INSERM U575; Physiopathologie du Système Nerveux; 5, rue Blaise Pascal; Strasbourg, France; Email: Lise.Roth@neurochem.u-strasbg.fr
Submitted: 08/28/08; Accepted: 09/09/08
Previously published online as a Cell Adhesion & Migration E-publication: http://www.landesbioscience.com/journals/celladhesion/article/6960

www.landesbioscience.com Cell Adhesion & Migration 217
NRP2 Mediates Tumoral and Adult Lymphangiogenesis

Considering these elements, the authors then asked the question: what could be NRP2 function in adult lymphatics? Expression analyses revealed no presence of NRP2 either in lymphatic vessels of colon, nor in lymph node in normal adult mice. Remarkably, they showed a strong expression of NRP2 in lymphatic vessels within and around tumors, and in lymph nodes adjacent to tumors. Moreover, NRP2 was expressed in new lymphatic vessels formed after VEGF-C application in the cornea. Thus, NRP2 appears as a mediator of both developmental and adult lymphangiogenesis, but does not seem to play a role in maintaining quiescent adult lymphatics. As NRP2 could be an interesting player in tumor metastasis, Bagri and collaborators next tested anti-NRP2B treatment on the formation of lung metastasis in two different tumor models. Orthotopic transplantation of 66c14 cells and heterotopic subcutaneous transplantation of C6 cells resulted in reproducible development of tumors and lung metastasis. The authors evaluated distant organ metastasis. In both cases, the anti-NRP2B treatment did not affect the primary tumor growth, but it caused a significant reduction in the average number of metastatic nodules per lung compared to control. Moreover, the antibody treatment resulted in a reduction of total metastatic volume in comparison to control treatment.

Anti-NRP2 B Treatment Blocks Lymphangiogenesis while Sparing Established Lymphatics

The comparison of primary tumors helped to determine the mechanism by which anti-NRP2B treatment leads to a decrease of metastatic nodules. PECAM-1 and LYVE-1 staining revealed a dramatic reduction of lymphatic vessel density and change of lymphatic morphology in anti-NRP2B-treated tumors compared to control tumors, whereas no change in blood vessel number or morphology was observed. These observations suggest that nodule formation decrease is due to the reduction of tumor-associated lymphatics induced by anti-NRP2B treatment. Two mechanisms could then explain tumor metastasis inhibition, either a disruption of existing lymphatics, or an inhibition of lymphangiogenesis. The analysis of anti-NRP2B-treated tumors revealed disrupted lymphatics in both early and late stage points, suggesting that anti-NRP2B rather inhibits tumor lymphangiogenesis. Moreover, anti-NRP2B treatment had no qualitative or quantitative effect on normal lymphatics in adult mice, demonstrating that blocking of NRP2 does not affect maintenance of quiescent adult lymphatics. Because anti-NRP2B could reduce total lymphatic density while sparing functional vessels, intradermal lymphangiography with Evans Blue was performed on control and anti-NRP2B-treated mice bearing C6 and 66s14 tumors before the onset of tumor necrosis. These analyses revealed a decrease in functional vessel formation in both tumor types compared to control, which would be responsible for the reduction of tumor metastasis. Finally, sentinel lymph nodes were analyzed for tumor cells after subcutaneous transplantation of C6 cells into mice ear. Tumor cells were identified in the SLN of control animals three days after implantation, whereas micrometastasis was only identified six days post-implantation in anti-NRP2B-treated animals. SLN is the first tissue that metastizing cells encounter after exiting the tumor and entering the lymphatic system. This analysis thus reveals that blocking NRP2 results in a delay of metastasis via the lymphatic system.

In this study, Bagri and colleagues identified an attractive novel target for the inhibition of tumor metastasis. This is of particular interest as metastasis accounts for much of the mortality associated with cancer and no clinical therapeutic strategy that specifically targets the development of metastasis has been established yet. Importantly, the authors demonstrated that the activation of NRP2 by VEGF-C mediates specific functional effects, at least partly independent from the enhancement of VEGFR activity (Fig. 1). Hence, as mentioned in the article, anti-NRP2B treatment does not affect normal lymphatics, which could avoid complications observed with VEGFR3 treatment, such as lymphedema.

NRP2 was first discovered as a receptor for axon guidance molecules, the semaphorins. Interestingly, a growing body of evidence tends to show the implication of semaphorins and neuropilins in angiogenesis and tumor progression. In particular, NRP1
NRP2 mediates adult and tumoral lymphangiogenesis

has been the focus of several studies on cancer biology. In a recent article, a model has been built where NRP1 modulates endothelial cell motility in response to VEGF as part of the receptor complex together with VEGFR-2, by binding additional molecule mediators.6 Thus, neuropilins, which were originally described as axon guidance molecule receptors, appear to play a pivotal role in the modulation of tumor growth and tumor metastasis. Hence, they join members of the slit, the netrin and the ephrin families that also exhibit dual activities as axon guidance factors and as modulators of angiogenesis and tumour progression. Several therapeutic strategies should be directed towards these new players in the years to come.

References
1. Chen Z, Varney ML, Backora MW, Cowan K, Selheim JC, Talmadge JE, et al. Downregulation of vascular endothelial cell growth factor-C expression using small interfering RNA vectors in mammary tumors inhibits tumor lymphangiogenesis and spontaneous metastasis and enhances survival. Cancer Res 2005; 65:9004-11.
2. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003; 9:669-76.
3. Favier B, Alam A, Barron P, Bonnin J, Laboudie P, Fons P, et al. Neuropilin-2 interacts with VEGFR-2 and VEGFR-3 and promotes human endothelial cell survival and migration. Blood 2006; 108:1243-50.
4. Caunt M, Mak J, Liang WC, Stawicki S, Pan Q, Tong RK, et al, Tessier-Lavigne M, Bagri A. Blocking neuropilin-2 function inhibits tumor cell metastasis. Cancer Cell 2008; 13:331-42.
5. Neufeld G, Kessler O. The semaphorins: versatile regulators of tumour progression and tumour angiogenesis. Nat Rev Cancer 2008; 8:632-45.
6. Pan Q, Chanthery Y, Liang WC, Stawicki S, Mak J, Rathore N, et al. Blocking neuropilin-1 function has an additive effect with anti-VEGF to inhibit tumor growth. Cancer Cell 2007; 11:53-67.