

Netrin-1 expression confers a selective advantage for tumor cell survival in metastatic breast cancer

Julien Fitamant*, Céline Guenebeaud*, Marie-May Coissieux*, Catherine Guix*, Isabelle Treilleux†, Jean-Yves Scoazec‡, Thomas Bachelot†, Agnès Bernet*, and Patrick Mehlen*§

*Apoptosis, Cancer, and Development Laboratory—Equipe Labellisée “La Ligue,” Centre National de la Recherche Scientifique, Unité Mixte de Recherche 5238, and †Institut National de la Santé et de la Recherche Médicale U590, Centre Léon Bérard, Université de Lyon 69008 Lyon, France; and ‡Institut National de la Santé et de la Recherche Médicale, U865 and Plate-forme d’Histopathologie du Petit Animal IFR62, Faculté Laennec, Université Claude Bernard, 69437 Lyon, France

Edited by Bert Vogelstein, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, and approved January 15, 2008 (received for review October 15, 2007)

Netrin-1, an axon navigation cue was proposed to play a crucial role during colorectal tumorigenesis by regulating apoptosis. The netrin-1 receptors DCC and UNC5H were shown to belong to the family of dependence receptors that share the ability to induce apoptosis in the absence of their ligands. Such a trait confers on these receptors a tumor suppressor activity. Expression of one of these dependence receptors at the surface of a tumor cell is indeed speculated to render this cell dependent on ligand availability for its survival, hence inhibiting uncontrolled cell proliferation or metastasis. Consequently, it is a selective advantage for a tumor cell to lose this dependence receptor activity, as previously described with losses of DCC and UNC5H expression in human cancers. However, the model predicts that a similar advantage may be obtained by gaining autocrine expression of the ligand. We describe here that, unlike human nonmetastatic breast tumors, a large fraction of metastatic breast cancers overexpress netrin-1. Moreover, we show that netrin-1-expressing mammary metastatic tumor cell lines undergo apoptosis when netrin-1 expression is experimentally decreased or when decoy soluble receptor ectodomains are added. Such treatments prevent metastasis formation both in a syngenic mouse model of lung colonization of a mammary cancer cell line and in a model of spontaneous lung metastasis of xenografted human breast tumor. Thus, netrin-1 expression observed in a large fraction of human metastatic breast tumors confers a selective advantage for tumor cell survival and potentially represents a promising target for alternative anticancer therapeutic strategies.

apoptosis | DCC | dependence receptor

Netrin-1, a diffusible laminin-related protein, has been shown to play a major role in the control of neuronal navigation during the development of the nervous system by interacting with its main receptors, DCC (Deleted in Colorectal Cancer) (1–3) and UNC5H (4, 5). However, more recently, netrin-1 has emerged as a completely different molecule that regulates cell survival. Indeed, the netrin-1 receptors DCC and UNC5H, i.e., UNC5H1, UNC5H2, and UNC5H3, belong to the so-called dependence receptor family (6, 7). Such receptors share the functional property of inducing cell death when disengaged from their ligands, whereas the presence of their ligands blocks this proapoptotic activity. Such receptors thus create cellular states of dependence on their respective ligands (8, 9).

This dependence effect has been suggested to act as a mechanism for eliminating tumor cells that would develop in settings of ligand unavailability: proliferation of tumor cells in a cell environment with constant and limited ligand presence or migration of metastatic tumor cells toward tissues where the ligand is not expressed. A selective advantage for a tumor cell would then be to lose the proapoptotic activity of its dependence receptors. Along this line, DCC was proposed in the early 1990s to be a tumor-suppressor gene, whose expression is lost in the vast majority of human cancers (10, 11). This hypothesis also fits with the observation that *UNC5H* genes are down-regulated in the vast majority of colorectal tumors,

hence suggesting that the loss of *UNC5H* genes represents a selective advantage for tumor development (12–14). Interestingly, in mice, both inactivation of *UNC5H3* and overexpression of *netrin-1* in the gastrointestinal tract are associated with intestinal tumor progression (13, 15), hence demonstrating *per se* that loss of netrin-1 dependence receptors in the human pathology is a causal factor for tumor progression (16).

However, the model described above predicts that not only loss of netrin-1 receptors but also gain of ligand expression, i.e., autocrine expression, should be observed in human cancers, because they should represent similar selective advantages for tumor progression. This question is important not only for basic knowledge but also for putative therapy: Indeed, triggering tumor-cell death by inhibiting the extracellular interaction between netrin-1/receptors could represent an appealing anticancer strategy. We show here that the majority of breast tumors with metastatic propensity show increased netrin-1 expression, a feature that we used in different mouse models to prevent/inhibit metastatic development.

Results

Netrin-1 Is a Marker for Human Metastatic Breast Cancer. We first analyzed by quantitative (Q)RT-PCR, expression of netrin-1, its dependence receptors, i.e., DCC, UNC5H2, and UNC5H3, and, as positive control, a known marker of metastasis, CXCR4 (17) in a panel of 51 breast primary tumors. These tumors were from lymph node-negative patients (N0, 16 patients), lymph node-positive patients (N + M0, 19 patients) or metastatic patients at the time of diagnosis (M+, 16 patients). As already described, CXCR4 is significantly more expressed in N + M0 or M+ primary tumors compared with N0 tumors [supporting information (SI) Fig. 5A in SI Appendix]. Although DCC was barely detectable and UNC5H expression failed to display significant changes between the different types of tumors (data not shown, Fig. 1A and SI Fig. 5B in SI Appendix), netrin-1 is significantly more expressed in N + M0 tumors than in N0 tumors (median, 1.8 versus 0.5; $P = 0.007$) with a range of netrin-1 expression higher in N + M0 tumors (Fig. 1A and SI Fig. 5B in SI Appendix). Whereas 31.5% of the N + M0 tumors show at least a 5-fold increase in netrin-1, no such increase was detected in any tested N0 tumors (Fig. 1A and SI Fig. 5B in SI Appendix). An even more striking difference is observed when comparing netrin-1 expression in M+ versus N0 tumors (median,

Author contributions: A.B. and P.M. contributed equally to this work; J.F., A.B., and P.M. designed research; J.F., C. Guenebeaud, M.-M.C., C. Guix, I.T., J.-Y.S., and A.B. performed research; I.T. and T.B. contributed new reagents/analytic tools; J.F., I.T., J.-Y.S., T.B., A.B., and P.M. analyzed data; and P.M. wrote the paper.

The authors declare no conflict of interest.

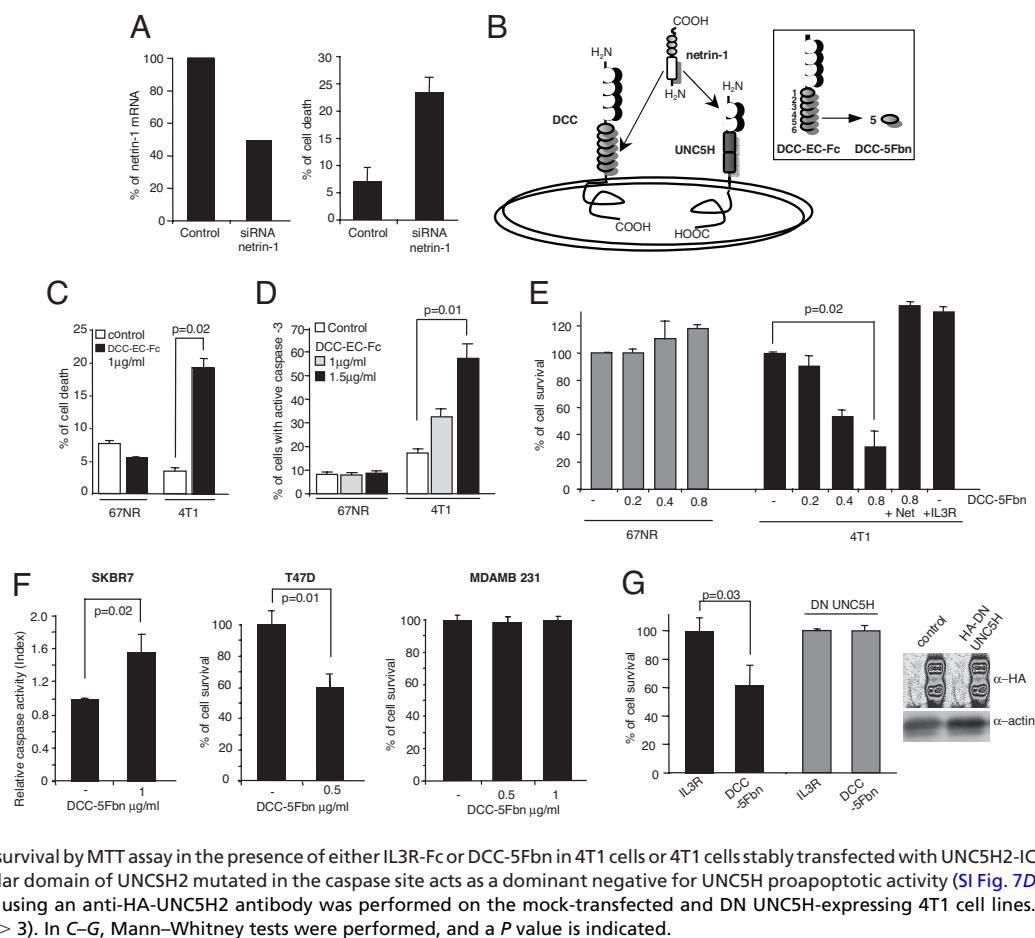
This article is a PNAS Direct Submission.

§To whom correspondence should be addressed. E-mail: mehlen@lyon.fnclcc.fr.

This article contains supporting information online at www.pnas.org/cgi/content/full/0709810105/DC1.

© 2008 by The National Academy of Sciences of the USA

Fig. 2. Induction of metastatic cell death by inhibiting the netrin-1 autocrine loop. (A) Induction of 4T1 cell death by reducing netrin-1 level. The 4T1 cells were treated with scramble siRNA (control) or netrin-1 siRNA. Twenty-four hours later, the netrin-1 mRNA level was quantified by QRT-PCR (Left), and cell death was determined 1 day later by trypan blue-exclusion assay (Right). (B) Scheme representing netrin-1 and its receptors, DCC and UNC5H. (Inset) The extracellular part of the DCC receptor (DCC-EC-Fc) and the fifth fibronectin type III domain of DCC (DCC-5Fbn) used to induce cell death. (C–E) Quantitative analysis of cell death in 67NR and 4T1 cells treated with DCC-EC-Fc (C and D), with DCC-5Fbn (E) or with an unrelated soluble protein IL3R-EC-Fc. Cell death/survival was quantified by the trypan blue-exclusion assay (C), by caspase activity measurement by flow cytometry (D), or by MTT assay (E). In this latter test, netrin-1 was added in excess to reverse the effect of DCC-5Fbn. (F) Cell-death induction by DCC-5Fbn in SKBR7 and T47D cell lines but not in MDAMB231 cells. Cell survival was quantified by MTT assay (Right), and apoptosis was measured by caspase activity assay (Left). (G) Quantitative analysis of cell survival by MTT assay in the presence of either IL3R-Fc or DCC-5Fbn in 4T1 cells or 4T1 cells stably transfected with UNC5H2-IC D412N (DN UNC5H; i.e., the intracellular domain of UNC5H2 mutated in the caspase site acts as a dominant negative for UNC5H proapoptotic activity (SI Fig. 7D in SI Appendix). (Inset) Western blot using an anti-HA-UNC5H2 antibody was performed on the mock-transfected and DN UNC5H-expressing 4T1 cell lines. Standard deviations are indicated ($n > 3$). In C–G, Mann–Whitney tests were performed, and a *P* value is indicated.



tumor, showing different metastatic potentials when injected into syngenic mice. Although 67NR cells form primary mammary tumors but no metastasis, 4T1 cells form primary tumors and metastasis, especially in the lung, the liver, and the bone marrow. Interestingly, although netrin-1 receptors (i.e., DCC, UNC5H1, UNC5H2, and UNC5H3) failed to show significant difference between 67NR and 4T1 cell lines, netrin-1 mRNA was hardly detected in 67NR cells, whereas it was expressed in 4T1 cells (Fig. 1DE and SI Fig. 6A in SI Appendix). This presence of the netrin-1 mRNA in 4T1 cells is associated with the detection of netrin-1 protein after netrin-1 immunostaining performed in the absence of cell permeabilization (Fig. 1E). Therefore, metastatic 4T1 cells show autocrine production of netrin-1.

We next investigated whether this netrin-1 autocrine production confers a selective advantage to 4T1 cells by inhibiting DCC/UNC5H-induced cell death. We first down-regulated expression of netrin-1 in 4T1 cells using RNAi strategy. As shown in Fig. 2A, down-regulation of netrin-1 in 4T1 cells was associated with cell-death induction monitored by a trypan blue-exclusion assay. As a second approach, we looked for a compound that may inhibit the interaction between netrin-1 and its receptors. It was reported that a domain located in the N terminus of netrin-1 (the so-called laminin-VI domain) interacts with both DCC and UNC5H receptors [Fig. 2B; (20)]. We show that a soluble extracellular domain of DCC (DCC-EC-Fc) is able to inhibit both DCC/netrin-1 and UNC5H2/netrin-1 interaction, as measured by ELISA (SI Fig. 7A in SI Appendix). This soluble ectodomain is also able to inhibit netrin-1-induced DCC-mediated signaling pathways [e.g., MAPK activation (3)] (SI Fig. 7B in SI Appendix). DCC-EC-Fc was then added to a 4T1 cells culture—or as a control 67NR cells culture—and cell death/survival was monitored, either by a trypan blue-

exclusion assay (Fig. 2C) or by a MTT assay (data not shown). In addition, apoptosis was more specifically monitored by measuring caspase activity (Fig. 2D). As shown in Fig. 2C and D, addition of the competing soluble protein in the culture medium triggers death of 4T1 cells, whereas it has no effect on 67NR cell death.

Because the complete extracellular domain of DCC appears as only of modest interest for use *in vivo* and in therapy (DCC-EC-Fc is $\approx 1,100$ aa large), we looked for an alternative polypeptide from the DCC extracellular domain that could trigger apoptosis in 4T1 cells but not in 67NR cells. We consequently produced a flag-tagged fifth fibronectin type III domain of DCC, DCC-5Fbn, a domain located in the ectodomain of DCC that is known to interact with netrin-1 (20) (Fig. 2B Inset). This 100-aa protein does not interfere with the binding of DCC/netrin-1 or UNC5H/netrin-1 but affects the ability of netrin-1 to trigger multimerization of these receptors, a multimerization required for the inhibitory activity of netrin-1 on DCC- or UNC5H-induced cell death (F. Mille, F. Llambi, C. Guix, C. Thibert, J.F., C. Guenebeaud, S. Castro-Oregon, D. E. Bredesen, and P.M., unpublished work). As shown in Fig. 2E, addition of DCC-5Fbn triggers, in a dose-dependent manner, death of 4T1 cells as measured by MTT assay. Similar results were obtained by using trypan blue-exclusion or caspase-activity assays (data not shown). This cell-death effect is directly associated with netrin-1 autocrine expression because (i) DCC-5Fbn has no effect on 67NR cells, (ii) addition of an excess netrin-1 blocks the death effect of DCC-5Fbn, and (iii) IL3R-EC-Fc (the extracellular domain of the IL3 receptor) fails to trigger 4T1 cell death (Fig. 2E).

The acquired survival advantage through netrin-1 autocrine expression is not restricted to murine tumor cells, because it is detected in human breast cancer cell lines. Indeed, addition of

rometastases were detected. As shown in Fig. 3J, DCC-5Fbn significantly decreases lung macrometastasis. The antimetastatic effect of DCC-5Fbn is, moreover, associated with increased mouse survival as shown in Fig. 3K.

As a second approach to evaluate the antimetastatic effect of DCC-5Fbn, we investigated whether spontaneous development of metastases in lung from a primary breast cancer could also be inhibited by DCC-5Fbn. We took advantage of a fresh human breast tumor, which was characterized as expressing a high level of netrin-1, and that, when xenografted in nude mice, was giving rise to spontaneous development of metastases in the lung. Once tumors reached an optimal size after xenograft in nude mice, [i.e., lung colonization was detected at this stage (data not shown)], mice were treated daily with either PBS or DCC-5Fbn. As shown in Fig. 4, DCC-5Fbn treatment is associated with a significant decrease in the number of aggressive metastases in lungs. Interestingly, this reduction in the number of metastases is associated with the detection of metastatic lesions that are much smaller and associated with dense inflammatory reactions (Fig. 4A and B), hence further supporting the view that, in mice, the inhibition by DCC-5Fbn of the pro-survival activity conferred by netrin-1 autocrine expression is associated with antimetastatic effect.

Discussion

Here, we show that netrin-1 expression may be considered as a marker of a breast tumor's ability to disseminate. Most of the breast primary tumors with metastasis abilities showed elevated netrin-1 expression. Both the human pathology and tumor cell line data and the mouse models described above support the view that this elevated netrin-1 level is a selective advantage acquired by the cancer cell to escape netrin-1-dependence receptor-induced apoptosis and, consequently, to survive independently of netrin-1 availability. From a mechanistic point of view, in human pathology, this autocrine expression of netrin-1 probably inhibits UNC5H-induced cell death. Along this line, the antimetastatic effect of netrin-1 siRNA in the 4T1 model is inhibited by injection of UNC5H1 and UNC5H2 siRNA (SI Fig. 8B in SI Appendix). Thus, DCC, which is barely detectable in the different groups (N0, N + M0, M+) of breast cancers studied, is probably of relatively modest interest in metastatic breast cancers. Thus, as predicted by the dependence receptor model, we have now shown that a tumor cell can escape receptor dependency in at least three ways. First,

expression of the dependence receptor can be down-regulated, as extensively described for DCC and more recently for UNC5H (10, 12–14, 21). Second, the downstream death signaling can be shut down. We have recently shown that UNC5H2 proapoptotic activity relies on the binding of UNC5H2 to the serine/threonine DAPK (22), a protein that was demonstrated to be involved in metastasis regulation and down-regulated in human malignancy (23). Here, we show that a third selective advantage for the tumor cell is the self-production of the dependency ligand. One intriguing question remains as to why breast tumors with metastatic propensity seem to have preferably selected netrin-1 self-production rather than receptor loss, whereas colorectal tumors have mostly selected loss of the receptors rather than gain of netrin-1 expression—indeed, only 7% of colorectal cancers show an increase of netrin-1 expression (13, 15). A possible explanation is that netrin-1 expression not only confers a gain in survival to the disseminating cells, but also possibly a gain in the nonapoptotic/positive signaling of netrin-1 receptors. It is important to note that netrin-1 was originally described as a guidance cue (24), which, even though completely unproven, could play a role in the tropism of metastatic cells. Other proposed roles of netrin-1 include adhesion and morphogenesis regulation (25–27), both mechanisms that may be of importance for metastasis development. Similarly, netrin-1 was recently proposed to play a role during embryonic angiogenesis, and, even though conflicting results have been published (28–32), we cannot, at this stage, rule out the role of netrin-1 as an angiogenic factor that somehow could favor metastasis development at the secondary site. However, the gain of “positive” signaling by netrin-1 autocrine expression is probably not sufficient *per se* to promote metastasis. Indeed, when netrin-1 was forced in the nonmetastatic 67NR cells, we failed to observe any significant increase of lung metastasis after 67NR injection into mammary glands (SI Fig. 6 in SI Appendix). Thus, netrin-1 expression in tumor cells is probably not sufficient to enable metastasis formation from the primary site but mainly confers survival to metastatic cells.

These observations not only provide evidence for the importance of ligand/dependence receptor pairs in the regulation of tumor development, but also suggest a putative therapeutic strategy. Indeed, as of today, there is no efficient treatment for patients with metastatic breast cancer, a lack of treatment that leads to the death of 400,000 women worldwide per year (33). Here, we propose that a treatment based on inhibition of the interaction between netrin-1 and its dependence receptors could positively effect a large fraction of the patients suffering from metastatic breast cancer, i.e., patients who would show high netrin-1 expression in primary tumors. Future preclinical and clinical work needs now to demonstrate whether netrin-1 could represent an appealing target in two-thirds of metastatic breast cancers.

Methods

A detailed methods section is provided in SI Materials and Methods in SI Appendix.

Cell Line, Transfection, Immunoblotting, Immunoprecipitation, Plasmid Constructs, siRNA, and Reagents. Human breast and mouse mammary cell lines listed were cultured by using standard procedures and were obtained from D. Birnbaum (Institut National de la Santé et de la Recherche Scientifique U 599, Centre de Recherche, Marseille, France) and F. Miller (Wayne State University, Detroit). Transfection, immunoblotting, and immunoprecipitation were performed as described in SI Materials and Methods in SI Appendix and essentially as described (22). The different constructs are described in SI Materials and Methods in SI Appendix. For cell culture use, scramble and netrin-1 siRNAs were designed by Santa Cruz Biotechnology as a pool of three target-specific 20- to 25-nt siRNAs. For the *in vivo* approach, two different single netrin-1, DCC, UNC5H1, or UNC5H2 siRNAs were produced (Sigma-Proligo). DCC-5Fbn was produced upon expression in BL21 lysate and upon affinity chromatography using Flag-Sepharose (Sigma). DCC-EC-Fc was obtained from R & D Systems and Netrin-1, UNC5H2-EC-Fc, GST-DCC-5Fbn, and GST-FaDD from Apotech/Axxora.

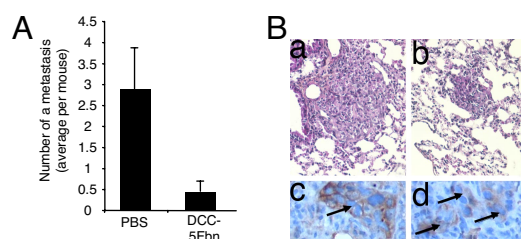


Fig. 4. Disruption of the netrin-1 autocrine loop inhibits spontaneous lung metastasis from a xenografted human breast tumor. (A) Antitumor effect of DCC-5Fbn in nude mice xenografted with a fresh human breast tumor. A human breast tumor showing a high netrin-1 level was xenografted into nude mice, and when the tumor reached a palpable size, the mice were *i.v.* treated daily with PBS or DCC-5Fbn. Metastases were analyzed and enumerated by a pathologist. (B) Comparative low-power magnifications of lung metastases in control (a) and DCC-5Fbn-treated (b) animals; tumor cells, characterized by large size, abundant cytoplasm, and irregular nucleus, are arranged in small masses of variable diameter, scattered within the lung parenchyma. (c and d) Higher magnifications of lung metastases in a DCC-5Fbn-treated animal. Metastatic cells, revealed by an anti-human cytokeratin 7 antibody, form a small mass surrounded by a dense inflammatory reaction (c); at an advanced stage, tumor cells are dissociated by infiltrative inflammatory cells (d). (a and b) H&E staining, original magnifications $\times 200$. (c and d) Immunoperoxidase, followed by nuclear counterstaining with Mayer's hematoxylin, original magnification $\times 400$.

Human Breast Tumor Samples, QRT-PCR, and Immunohistology. Fifty-one human breast cancer samples were provided by the tumor bank of the Centre Léon Bérard. Fresh tissue of the tumor was obtained during breast surgery before any systemic therapy and snap-frozen in liquid nitrogen. QRT-PCR was performed as described in *SI Materials and Methods in SI Appendix* and essentially as described (13). The sequences of the primers used are available upon request. Paraffin-embedded tumors tissues fixed in formalin were used for analysis. The pathologist selected representative areas from breast carcinomas ($N + M0, M+$) or liver or uterine metastases. Immunostaining was performed as described in *SI Materials and Methods in SI Appendix* and essentially as described (13).

Cell Death Assays. The different cell death assays (trypan blue exclusion, MTT, caspase-3 activity assay) were performed as described in the supplementary materials and essentially as described (22, 34).

Tumor Mouse Models. A complete description of the mouse models is provided in *SI Materials and Methods in SI Appendix*. Briefly, for mammary gland injection of 67NR cells, 10^6 cells were injected. For i.v. injection, 10^5 (unless indicated) 4T1-luc cells were injected into a tail vein, and mice were either killed or analyzed by using luminescence recording. Lungs were removed, and metastatic nodules were counted and histological classification and grading of neoplastic lesions was

performed in a blinded fashion and according to standard procedures. Daily i.p. injection of scramble siRNA or netrin-1 siRNA ($4 \mu\text{g}$ per mouse) (or DCC, UNC5H1, or UNC5H2) were performed according to the procedure established by SeleXel Corp. DCC-5Fbn treatments were performed according to the Fig. 3 legend. Xenografts of fresh human breast tumors in nude mice were performed according to the protocol developed by Xentech. Among 20 fresh breast tumors, the tumor BC174 expressing high levels of netrin-1 and of UNC5H receptors was selected. For each xenograft, when tumors reached 62 mm^3 , PBS or $1.25 \mu\text{g}$ per gram of body weight DCC-5Fbn were administered i.v. every day.

ACKNOWLEDGMENTS. We thank O. Donze (Apotech, Lausanne, Switzerland) for advice and reagents; F. Cabon, I. Puisieux, and C. Delloye for technical advice; J. Blachier, A. Potemski, I. Durand, N. Gadot, and S. Goddard-Leon for excellent technical help; F. Miller, J. Adélaïde [Institut National de la Santé et de la Recherche Scientifique (INSERM) U 599, Marseille, France] C. Caux, D. Birnbaum (INSERM U 590, Lyon, France), and G. Grelier (INSERM U 590, Lyon, France) for materials; H. Bilak for text correction; A. Puisieux for helpful discussion; Xentech for human breast tumor xenografts; and SeleXel for *in vivo* siRNA. This work was supported by an institutional grant from the Centre National de la Recherche Scientifique, Centre Léon Bérard, University of Lyon, and from the Ligue Contre le Cancer, Institut National du Cancer, Agence Nationale de la Recherche Blanche, and European Union Specific Targeted Research Project Hermione.

- Serafini T, et al. (1996) Netrin-1 is required for commissural axon guidance in the developing vertebrate nervous system. *Cell* 87:1001–1014.
- Keino-Masu K, et al. (1996) Deleted in Colorectal Cancer (DCC) encodes a netrin receptor. *Cell* 87:175–185.
- Forcet C, et al. (2002) Netrin-1-mediated axon outgrowth requires deleted in colorectal cancer-dependent MAPK activation. *Nature* 417:443–447.
- Ackerman SL, et al. (1997) The mouse rostral cerebellar malformation gene encodes an UNC-5-like protein. *Nature* 386:838–842.
- Hong K, et al. (1999) A ligand-gated association between cytoplasmic domains of UNC5 and DCC family receptors converts netrin-induced growth cone attraction to repulsion. *Cell* 97:927–941.
- Mehlen P, et al. (1998) The DCC gene product induces apoptosis by a mechanism requiring receptor proteolysis. *Nature* 395:801–804.
- Llambi F, Causeret F, Bloch-Gallego E, Mehlen P (2001) Netrin-1 acts as a survival factor via its receptors UNC5H and DCC. *EMBO J* 20:2715–2722.
- Mehlen P, Thibert C (2004) Dependence receptors: between life and death. *Cell Mol Life Sci* 61:1854–1866.
- Bredesen DE, Mehlen P, Rabizadeh S (2005) Receptors that mediate cellular dependence. *Cell Death Differ* 12:1031–1043.
- Fearon ER, et al. (1990) Identification of a chromosome 18q gene that is altered in colorectal cancers. *Science* 247:49–56.
- Kinzler KW, Vogelstein B (1996) Lessons from hereditary colorectal cancer. *Cell* 87:159–170.
- Thiebault K, et al. (2003) The netrin-1 receptors UNC5H are putative tumor suppressors controlling cell death commitment. *Proc Natl Acad Sci USA* 100:4173–4178.
- Bernet A, et al. (2007) Inactivation of the UNC5C Netrin-1 receptor is associated with tumor progression in colorectal malignancies. *Gastroenterology* 133:1840–1848.
- Shin SK, et al. (2007) Epigenetic and genetic alterations in Netrin-1 receptors UNC5C and DCC in human colon cancer. *Gastroenterology* 133:1849–1857.
- Mazelin L, et al. (2004) Netrin-1 controls colorectal tumorigenesis by regulating apoptosis. *Nature* 431:80–84.
- Grady WM (2007) Making the case for DCC, UNC5C as tumor-suppressor genes in the colon. *Gastroenterology* 133:2045–2049.
- Muller A, et al. (2001) Involvement of chemokine receptors in breast cancer metastasis. *Nature* 410:50–56.
- van 't Veer LJ, et al. (2002) Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 415:530–536.
- Aslakson CJ, Miller FR (1992) Selective events in the metastatic process defined by analysis of the sequential dissemination of subpopulations of a mouse mammary tumor. *Cancer Res* 52:1399–1405.
- Geisbrecht BV, Dowd KA, Barfield RW, Longo PA, Leahy DJ (2003) Netrin binds discrete subdomains of DCC, UNC5 and mediates interactions between DCC, heparin. *J Biol Chem* 278:32561–32568.
- Mehlen P, Fearon ER (2004) Role of the dependence receptor DCC in colorectal cancer pathogenesis. *J Clin Oncol* 22:3420–3428.
- Llambi F, et al. (2005) The dependence receptor UNC5H2 mediates apoptosis through DAP-kinase. *EMBO J* 24:1192–1201.
- Inbal B, et al. (1997) DAP kinase links the control of apoptosis to metastasis. *Nature* 390:180–184.
- Serafini T, et al. (1994) The netrins define a family of axon outgrowth-promoting proteins homologous to *C. elegans* UNC-6. *Cell* 78:409–424.
- Yebra M, et al. (2003) Recognition of the neural chemoattractant Netrin-1 by integrins alpha6beta4 and alpha3beta1 regulates epithelial cell adhesion and migration. *Dev Cell* 5:695–707.
- Srinivasan K, Strickland P, Valdes A, Shin GC, Hinck L (2003) Netrin-1/neogenin interaction stabilizes multipotent progenitor cap cells during mammary gland morphogenesis. *Dev Cell* 4:371–382.
- Liu Y, et al. (2004) Novel role for Netrins in regulating epithelial behavior during lung branching morphogenesis. *Curr Biol* 14:897–905.
- Park KW, et al. (2004) The axonal attractant Netrin-1 is an angiogenic factor. *Proc Natl Acad Sci USA* 101:16210–16215.
- Lu X, et al. (2004) The netrin receptor UNC5B mediates guidance events controlling morphogenesis of the vascular system. *Nature* 432:179–186.
- Nguyen A, Cai H (2006) Netrin-1 induces angiogenesis via a DCC-dependent ERK1/2-eNOS feed-forward mechanism. *Proc Natl Acad Sci USA* 103:6530–6535.
- Wilson BD, et al. (2006) Netrins promote developmental and therapeutic angiogenesis. *Science*.
- Larrivee B, et al. (2007) Activation of the UNC5B receptor by Netrin-1 inhibits sprouting angiogenesis. *Genes Dev* 21:2433–2447.
- Andre F, et al. (2004) Breast cancer with synchronous metastases: trends in survival during a 14-year period. *J Clin Oncol* 22:3302–3308.
- Tauszig-Delamasure S, et al. (2007) The TrkC receptor induces apoptosis when the dependence receptor notion meets the neurotrophin paradigm. *Proc Natl Acad Sci USA* 104:13361–13366.
- Latil A, et al. (2003) Quantification of expression of netrins, slits and their receptors in human prostate tumors. *Int J Cancer* 103:306–315.
- de Kok JB, et al. (2005) Normalization of gene expression measurements in tumor tissues: comparison of 13 endogenous control genes. *Lab Invest* 85:154–159.