The Shed Ectodomain of Nr-CAM Stimulates Cell Proliferation and Motility, and Confers Cell Transformation

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Abstract

Nr-CAM, a cell-cell adhesion molecule of the immunoglobulin-like cell adhesion molecule family, known for its function in neuronal outgrowth and guidance, was recently identified as a target gene of β-catenin signaling in human melanoma and colon carcinoma cells and tissue. Retrovirally mediated transduction of Nr-CAM into fibroblasts induces cell motility and tumorigenesis. We investigated the mechanisms by which Nr-CAM can confer properties related to tumor cell behavior and found that Nr-CAM expression in NIH3T3 cells protects cells from apoptosis in the absence of serum by constitutively activating the extracellular signal-regulated kinase and AKT signaling pathways. We detected a metalloprotease-mediated shedding of Nr-CAM into the culture medium of cells transfected with Nr-CAM, and of endogenous Nr-CAM in B16 melanoma cells. Conditioned medium and purified Nr-CAM-Fc fusion protein both enhanced cell motility, proliferation, and extracellular signal-regulated kinase and AKT activation. Moreover, Nr-CAM was found in complex with α/β integrins in melanoma cells, indicating that it can mediate, in addition to homophilic cell-cell adhesion, heterophilic adhesion with extracellular matrix receptors. Suppression of Nr-CAM levels by small interfering RNA in B16 melanoma inhibited the adhesive and tumorigenic capacities of these cells. Stable expression of the Nr-CAM ectodomain in NIH3T3 cells conferred cell transformation and tumorigenesis in mice, suggesting that the metalloprotease-mediated shedding of Nr-CAM is a principal route for promoting oncogenesis by Nr-CAM. (Cancer Res 2005; 65(24): 11605-12)

Introduction

The development of human cancer is a multistage process involving genetic changes endowing tumor cells with both proliferative advantage and, at later stages, with altered adhesive and motile properties enabling invasion and metastasis. These cellular processes often include aberrant activation of genes normally expressed in other tissues in the course of various cellular processes in melanoma cells. The Nr-CAM ectodomain was found to be shed from the cell surface by metalloprotease-like activities, and this shed domain activated various signaling pathways, enhanced cell motility, and conferred tumorigenesis in mice. Nr-CAM was detected in a complex with integrins, suggesting that it also acts by paracrine mechanisms, by binding to integrins on neighboring cells. These properties of Nr-CAM make it a suitable marker for cancer diagnosis and a target for anticancer therapy.

Materials and Methods

Cell lines, cell growth, and wound healing. 293T, NIH3T3, and Chinese hamster ovary (CHO) cells were cultured in DMEM with 10% bovine calf serum and B16-M2R melanoma in DMEM/F12 (1:1 dilution) with 10% FCS and 1% glutamine. Conditioned medium was collected from confluent B16-M2R, or transfected 293T cells after incubation in serum-free medium for 48 hours, and was used for culturing NIH3T3 cells. Proteins from conditioned medium were precipitated with ethanol and proteins from the medium of an equal number of cells were analyzed by Western blotting. Cells were also incubated with GM6001 (20-40 μmol/L) and leupeptin (20-40 μmol/L) from Calbiochem (San Diego, CA) in serum-free medium for 24 hours before cell harvesting. Purified Nr-CAM-Fc fusion proteins were prepared as described (16) and added to cell cultures at different concentrations.

For cell growth analyses, the number of cells plated into 24-well dishes was determined every 24 hours in quadruplicates. In wound healing experiments, a “wound” was introduced into confluent monolayers of NIH3T3 or CHO cells preincubated overnight with mitomycin C (5 μg/mL).
Figure 1. Nr-CAM expression protects NIH3T3 cells from death induced by serum starvation. A, $5 \times 10^5$ cells expressing Nr-CAM, the puro^r gene (Puro), and the parental cell line (3T3) were seeded in the presence of 0.5% and 10% FCS, and the cultures were photographed after 4 days. B, cell number was determined after 12 hours and 4 days. C, Western blot analysis of cell lysates using antibodies against P-ERK, P-AKT, and total ERK and AKT of cells cultured for 12 hours in 0.5% serum and then stimulated for 6 hours with 0%, 0.5%, or 1.5% FCS.

Figure 2. The extracellular domain of Nr-CAM is shed into the culture medium. A, schematic representation of VSV-tagged Nr-CAM depicting its immunoglobulin domains (Ig) and fibronectin III (Fn III)-like repeats. The proposed cleaved domains of Nr-CAM obtained after Nr-CAM-VSV transfection into 293 cells produces a fragment comprised of the extracellular domain (ECD) and a segment containing the fibronectin repeats, the transmembrane region, and the cytoplasmic tail (ICD). B, 293 cells were transfected with increasing amounts of Nr-CAM-VSV plasmid and cell lysates were analyzed with different antibodies. Anti-VSV antibody recognized the full-length (FL) and ICD (top). ICD was also recognized by antibody 894 against the cytoplasmic tail of Nr-CAM (bottom). Antibody 837 recognized the full-length molecule and the cleaved extracellular domain (ECD, middle). C, conditioned medium from these cells contained the ectodomain of Nr-CAM that was detected by antibody 837 (ECD, bottom), but not by anti-VSV antibody (top). D, conditioned medium of B16-M2R melanoma cells, and E, NIH3T3 cells retrovirally transduced with Nr-CAM contained the shed ectodomain of Nr-CAM. Western blots of proteins shed into the medium (after 1 and 2 days) were stained with Ponceau to reveal their pattern before immunoblotting with anti-Nr-CAM antibody. F, shedding of Nr-CAM into the culture medium of B16-M2R melanoma cells, and G, NIH3T3-expressing Nr-CAM, was inhibited by the metalloprotease inhibitor GM 6001 (GM), but not by leupeptin (Leu). The level of cell-associated Nr-CAM and tubulin were determined from an equivalent number of cells.
to inhibit cell proliferation, as described (4). Photographs of the wounded areas were taken after staining cells with Giemsa.

Cell attachment and aggregation. B16-M2R cells expressing the puro gene and Nr-CAM siRNAs were trypsinized, and 10^4 cells were plated into 12-well dishes. The number of adherent cells after incubation at 37 ºC for 10, 20, 30, and 120 minutes was determined. In cell-cell aggregation assays, Nr-CAM-expressing NIH3T3 cells incubated with this conditioned medium was determined during 6 days. The motility of NIH3T3 cells (C) and CHO cells (D) was determined by introducing an artificial wound in a confluent monolayer of cells previously incubated (overnight) with conditioned medium from 293T cells transfected with Nr-CAM, or with empty vector (Control). Pictures were taken 16 hours after wounding using ×10 (C) and ×4 (D) lenses.

Plasmids, transfections, and retroviral infections. Transient transfection of 293T cells by the calcium phosphate method and retroviral infections were conducted as described (17) using the pBABE puromycin vectors. Cell cultures were incubated for 7 days with 10 μg/mL puromycin. Full-length human Nr-CAM cDNA, KIAA0343 in pBsk+/C0 vectors. Cell cultures were incubated for 7 days with 10 μg/mL puromycin were selected.

Coimmunoprecipitation and Western blotting. Coimmunoprecipitations and Western blotting using antibodies against mouse α5 (BMA5) and α4 integrin (R1-2) provided by Dr. B. Chan (University of Ontario, Canada), α4 (DE96741), and β1 integrin from Dr. P. Altevogt (German Cancer Research Center, Heidelberg, Germany), and antibody 837 against the extracellular domain of Nr-CAM (a gift from Dr. M. Grumet, Rutgers, State University of New Jersey, Piscataway, NJ), were carried out as described (18). The antibody against the intracellular domain of mouse Nr-CAM (no. 894) was provided by Dr. M. Grumet, antitubulin, extracellular signal-regulated kinase (ERK), and P-ERK were from Sigma (Nes Ziona, Israel), anti-AKT was from BD Transduction Laboratories (Franklin Lakes, NJ), anti-P-AKT from Cell Signaling Technologies (Beverly, MA), and anti-VSV was a gift from Dr. J.C. Perriard (Swiss Federal Institute of Technology, Zurich, Switzerland). Western blots were developed using the enhanced chemiluminescence method (Amersham, Buckinghamshire, United Kingdom).

Tumorigenicity assays. NIH3T3 cells (10^5 cells/mouse) expressing retrovirally transduced full-length Nr-CAM, ΔICD, or the empty vector, were injected s.c. into 6-week-old CD1 nude male mice. Groups of five mice were followed for 2 to 3 weeks until the size of tumors reached 1 to 2 cm in diameter. The tumors were excised and weighed after the animals were sacrificed according to the USPHS Policy on Humane Care and Use of Laboratory Animals.

Results

Nr-CAM expression in fibroblasts inhibits cell death induced by serum starvation and activates the mitogen-activated protein kinase/extracellular signal-regulated kinase and AKT pathways. Retrovirally-mediated transduction of Nr-CAM into NIH3T3 fibroblasts was previously shown to confer growth advantage on these cells (4). To determine whether the increased growth results from decreased dependence on serum factors, NIH3T3 cells, and cells expressing either the empty retroviral vector (Fig. 1A, Puro), or a vector containing Nr-CAM, were cultured in low serum concentrations (0.5%). After 4 days, Nr-CAM-expressing 3T3 cells survived this starvation, were spread on the substrate (Fig. 1A, Nr-CAM), and their number increased (Fig. 1B, left, black columns), whereas control 3T3 and puro 3T3 cells were round (Fig. 1A) and the majority of cells died, as evident from the decrease in cell number compared with 12 hours (Fig. 1B, white columns). In 10% serum, all three cell lines proliferated (Fig. 1B, right). We compared the levels of activated ERK (P-ERK) and AKT (P-AKT), 12 hours after incubation in 0.5% serum, because cell number was similar in the different cell lines at this time (Fig. 1B, white columns), and found that P-ERK and P-AKT were present in Nr-CAM-expressing cells, even in the absence of serum (Fig. 1C, lane 4), but not in puro NIH3T3 cells that required the presence of at least 1.5% serum for the induction of these pathways (Fig. C, lanes 1-3). This suggests that Nr-CAM expression in NIH3T3 cells could activate signaling pathways required for cell growth and survival, and enable cell proliferation in low serum concentrations.

The ectodomain of Nr-CAM is shed from the cell surface by metalloproteases. The ectodomain of L1, a close homologue of Nr-CAM, is shed from the surface of various human cancer cells and promotes cell motility (19). In the third fibronectin domain of Nr-CAM, there is a predicted cleavage site for furin and
trypsin (7, 20), suggesting that Nr-CAM, like L1, can be cleaved into two fragments: one containing most of the ectodomain (Fig. 2A, ECD) and the other comprising a small part of the extracellular, transmembrane, and intracellular domains (Fig. 2A, ICD). To examine whether Nr-CAM is cleaved and shed by cells, we constructed Nr-CAM tagged by VSV at the COOH-terminus (Fig. 2A) and transfected this cDNA into 293 cells. Anti-VSV antibody recognized the full-length molecule (~220 kDa) and a fragment containing the transmembrane and intracellular domains (ICD) that were VSV-tagged (~60 kDa; Fig. 2B, top). An antibody against the extracellular domain of Nr-CAM (antibody 837) detected the cleaved ectodomain (ECD) and the full-length molecule (Fig. 2B, middle). The antibody against the intracellular domain (ICD; antibody 894) recognized a segment containing the transmembrane and intracellular domains (Fig. 2B, bottom), but not the full-length molecule. This might result from diminished levels of full-length Nr-CAM compared with ICD in these cells, or the low affinity of this antibody.

We also analyzed the presence of shed Nr-CAM in conditioned medium of 293 cells transfected with Nr-CAM-VSV and detected the cleaved ectodomain of Nr-CAM in the medium using the 837 antibody recognizing the extracellular Nr-CAM segment (Fig. 2C, bottom, ECD). Endogenous Nr-CAM expressed in B16-M2R melanoma cells was also shed and we found high levels of Nr-CAM in their culture medium (Fig. 2D). The same was true for NIH3T3 cells expressing retrovirally transduced Nr-CAM (Fig. 2E). Shedding of endogenous melanoma cell Nr-CAM was inhibited by the metalloprotease inhibitor GM 6001 (GM), but not by leupeptin (Leu), an inhibitor of trypsin-like proteases (Fig. 2F). GM 6001 also inhibited Nr-CAM shedding by NIH3T3 cells stably transfected with Nr-CAM (Fig. 2G). These results indicate that Nr-CAM ectodomain shedding in fibroblasts and melanoma cells is regulated by metalloproteases.

**Conditioned medium containing shed Nr-CAM induces cell proliferation and motility, and activates extracellular signal-regulated kinase and AKT.** We wished to determine whether the shed Nr-CAM ectodomain has biological activity related to the role of Nr-CAM in cell growth and motility. Conditioned medium from 293T cells transfected with Nr-CAM, or Nr-CAM-Fc fusion constructs (Nr-Fc), contained extracellular derivatives of these molecules (Fig. 3A). When added to NIH3T3 cells, the cells proliferated faster in the presence of medium containing shed Nr-CAM (Fig. 3B). In addition, both NIH3T3 and CHO cells were more efficient in closing an artificial wound introduced into a monolayer (Fig. 3C and D, bottom) in the presence of conditioned medium containing shed Nr-CAM, compared with medium from cells transfected with empty vector (Fig. 3C and D, top).

We examined the possible contribution of the cytoplasmic domain of Nr-CAM to these functions of Nr-CAM and found that deletion mutants lacking the intracellular segment (ΔICD), or only the COOH-terminal PDZ-binding domain (ΔPDZ), were also shed into the medium (Fig. 3A, bottom). ΔICD was more efficiently shed than full-length Nr-CAM (Fig. 3A, compare

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**Figure 4.** The ectodomain of Nr-CAM activates MAPK/ERK, induces cell growth and motility, and binds to integrins. A, 293T cells were transfected with full-length (FL) and Nr-CAM mutants lacking the intracellular domain (ΔICD) or the PDZ binding domain (ΔPDZ), and their shedding into the culture medium was determined. B, the ability of medium containing the different mutant Nr-CAMs to promote cell growth, and C, MAPK and AKT activation in NIH3T3 cells was determined and compared with that using 10% FCS. D, purified Nr-CAM-Fc was incubated with serum-starved NIH3T3 cells and induction of cell proliferation in 0.5% serum was determined. E, the motility of these cells into a wound, when incubated with Nr-CAM-Fc fusion protein, was compared with conditioned medium (F) of 293T cells transfected with Nr-CAM-Fc plasmid. G, activation of ERK (P-ERK) was determined by incubation of serum-starved NIH3T3 cells with increasing concentrations of Nr-CAM-Fc protein, and with 50 ng EGF or 10% FCS. H, B16-M2R melanoma cell lysates were immunoprecipitated (IP) with either antibodies to Nr-CAM (lane 1), to α4 integrin (lane 2), or to green fluorescent protein (lane 3), and the precipitated proteins were analyzed by Western blotting with antibodies (IB) to Nr-CAM and β1 integrin.

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medium of cells, NIH3T3 cells were incubated with the purified ectodomain of Nr-CAM fused to Fc (Nr-CAM-Fc). The results showed that Nr-CAM-Fc fusion protein enhances the growth of NIH3T3 cells in 0.5% serum (Fig. 4D), and the rate of wound closure by these cells was faster (Fig. 4E), as also observed with conditioned medium of 293T cells transfected with Nr-CAM-Fc cDNA (Fig. 4F). The Nr-CAM-Fc protein also induced ERK activation to a level similar to that seen with 50 ng of epidermal growth factor (EGF; Fig. 4G, compare lanes 9-13).

Next, we wished to test whether the shed Nr-CAM ectodomain can bind to cell surface molecules other than Nr-CAM. Because the extracellular part of the closely related L1 molecule functions as a ligand activating integrins (19, 21), we examined whether Nr-CAM interacts with integrins expressed by melanoma cells. Coimmunoprecipitation experiments using B16-M2R melanoma cells with antibodies to α1 integrin and to Nr-CAM showed that Nr-CAM is in a complex that coprecipitated with α1 integrin (Fig. 4H, lane 2, top). This complex also contained β1 integrin (Fig. 4H, lane 2, bottom). In reverse coimmunoprecipitations with anti-Nr-CAM antibody, β1 integrin coprecipitated with Nr-CAM (Fig. 4H, lane 1, bottom). Nr-CAM did not precipitate with α5 integrin antibody, although B16 melanoma cells express α5 integrin (data not shown).

Expression of the Nr-CAM ectodomain in NIH3T3 cells confers cell transformation and tumorigenesis in mice. Because conditioned medium containing shed Nr-CAM ectodomain, or Nr-CAM-Fc fusion protein, induced NIH3T3 cell proliferation, we asked whether the stable expression of Nr-CAM lacking the cytoplasmic segment affects the properties of NIH3T3 cells. Retroviral transduction of NIH3T3 cells with Nr-CAM lacking the intracellular domain (ΔICD) conferred the ability to form foci by these cells when grown to high density, similar to the effect of full-length Nr-CAM expression in these cells (Fig. 5A). When ΔICD-expressing cells were injected into nude mice, they formed tumors, but these were smaller than those formed by cells transduced with full-length Nr-CAM (Fig. 5B and C). Expression of Nr-CAM also increased ERK phosphorylation in these cells (Fig. 5D, P-ERK), and ΔICD was shed into the culture medium by metalloproteases whose activity was inhibited by GM 6001 (Fig. 5E).

Suppression of Nr-CAM by small interfering RNA inhibits cell-substrate and cell-cell adhesion, and the tumorigenicity of melanoma cells. We wished to determine whether endogenous Nr-CAM expression in melanoma cells is necessary to maintain their adhesive and tumorigenic capacities. For this, we expressed siRNA constructs to Nr-CAM, using retroviral vectors, and isolated B16-M2R cells stably displaying diminished levels of Nr-CAM (Fig. 6A). Analysis of the initial adhesion capacity of such cells to the substrate, and their cell-cell aggregation, revealed that both were reduced when different clones of B16-M2R cells with suppressed Nr-CAM were compared with control cells (Fig. 6C and D). Moreover, the ability of B16-M2R cells with reduced Nr-CAM to form tumors in nude mice was dramatically inhibited (Fig. 6B). These results show that Nr-CAM expression by melanoma cells plays a necessary role in determining both their adhesive and tumorigenic capacities.

Discussion

Activation of proliferative and antiapoptotic pathways by Nr-CAM. This study showed that Nr-CAM expression plays a key role in cell transformation and tumorigenesis. Nr-CAM expression in NIH3T3 cells enhanced cell proliferation and motility, and induced ERK and AKT phosphorylation. The extracellular domain of Nr-CAM induces cell proliferation and motility, and forms a complex with integrins. To determine whether this increased cell proliferation and motility is directly caused by the Nr-CAM ectodomain present in conditioned medium or if it is due to the release of a soluble factor, Nr-CAM-Fc fusion protein was used. The results showed that Nr-CAM-Fc fusion protein enhanced the growth of NIH3T3 cells over a period of 4 days, and induced ERK and AKT phosphorylation. These results indicate that Nr-CAM expression by melanoma cells directly caused by the Nr-CAM ectodomain present in conditioned medium of cells, NIH3T3 cells were incubated with the purified ectodomain of Nr-CAM fused to Fc (Nr-CAM-Fc). The results showed that Nr-CAM-Fc fusion protein enhances the growth of NIH3T3 cells in 0.5% serum (Fig. 4D), and the rate of wound closure by these cells was faster (Fig. 4E), as also observed with conditioned medium of 293T cells transfected with Nr-CAM-Fc cDNA (Fig. 4F). The Nr-CAM-Fc protein also induced ERK activation to a level similar to that seen with 50 ng of epidermal growth factor (EGF; Fig. 4G, compare lanes 9-13).

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role in determining the adhesive and tumorigenic abilities of melanoma cells, because siRNA-mediated suppression of Nr-CAM resulted in impaired cell-substrate and cell-cell adhesion, and a decreased capacity to form tumors in mice. In NIH3T3 cells, Nr-CAM expression enabled cell survival under serum starvation conditions by conferring constitutive activation of signaling pathways that induce cell growth [mitogen-activated protein kinase (MAPK)/ERK], or are antiapoptotic (AKT). Forced expression of the close homologue, L1, was similarly shown to activate ERK in NIH3T3 cells (22, 23), suggesting that Nr-CAM and other members of this family exert their effects in both neuronal cells (24, 25) and cancer cells by inducing these signaling pathways.

Regulation of Nr-CAM function by metalloproteases. An increasing number of soluble, “secreted” proteins are now recognized as being derived from integral membrane proteins that are cleaved by metalloproteases, resulting in the release of their ectodomain into the medium. These proteins are diverse in structure and function and comprise, among others, cell adhesion receptors of the cadherin family (26, 27). We have shown that the ectodomain of Nr-CAM (another adhesion receptor) is shed into the culture medium of melanoma cells by a process involving metalloproteases. Similarly, the ectodomain of L1 is shed by the metalloprotease ADAM10 (28, 29). Shedding of L1 promotes the migration of various human carcinoma cells on extracellular matrix components (19). We

Figure 6. Suppression of Nr-CAM by siRNA inhibits the adhesive and tumorigenic capacities of B16-M2R melanoma cells. A, B16-M2R cells were stably transfected with siRNA constructs to Nr-CAM and clones stably displaying reduced levels of Nr-CAM were isolated. B, the ability of such melanoma cell clones to form tumors was determined by their injection into nude mice and comparing tumor weight after 3 weeks. C, the initial adhesion to the substrate of these different cell clones was determined at early times after plating on culture dishes. D, the kinetics of cell-cell aggregation formation under gyration conditions at 37°C was determined.

Figure 7. Hypothetical models for the action of Nr-CAM. According to one possibility, Nr-CAM expression induced by β-catenin (β)-mediated transactivation of the Nr-CAM gene (4) results in Nr-CAM expression on the cell surface, its shedding into the medium by metalloproteases (MMP), and binding of the shed Nr-CAM to either integrins, or to Nr-CAM on the surface of the same cells (autocrine) to promote adhesion and activate various signaling pathways (ERK, AKT). In addition, the shed Nr-CAM may also bind to integrins on the surface of cells not expressing Nr-CAM, and activate signaling in these cells by paracrine mechanisms.
found that the culture medium containing the shed Nr-CAM ectodomain from transfected cells, and purified Nr-CAM-Fc fusion protein, both induced activation of ERK and AKT and enhanced cell proliferation and motility. Moreover, we showed that retroviral transduction of a cDNA containing the Nr-CAM extracellular domain, but lacking the cytoplasmic segment, conferred enhanced cell motility, cell transformation, and tumorigenesis in NIH3T3 cells. This suggests that metalloprotease-mediated cleavage of the Nr-CAM ectodomain produces a Nr-CAM molecular segment sufficient for conferring various properties associated with the development of tumorigenic capacity. It is noteworthy that several metalloproteases are transcriptional targets of β-catenin signaling (30–33). The coordinated activation of target genes including both Nr-CAM and metalloproteases that cleave its ectodomain, might be responsible for the functions of Nr-CAM in tumor development. Nr-CAM and L1 were recently shown to be target genes of the β-catenin-TCF complex in human cancer (4, 23), suggesting that activation of these genes is significant in tumor progression. This view is supported by a recent study showing that both L1 and ADAM10, the metalloprotease involved in its cleavage, are coregulated in cultured colon cancer cells, and are exclusively colocalized at the invasive front of human colorectal tumor tissue (23).

**Nr-CAM as a ligand for integrins.** Analysis of the Nr-CAM protein sequence revealed that, unlike its homologue L1 (34), Nr-CAM does not contain an RGD sequence in its ectodomain that could explain its association with integrins. Nevertheless, Nr-CAM coprecipitated with α4 and β1 integrins using antibodies against α4 integrin, and in reverse coimmunoprecipitations with antibodies against N-CAM, these integrins coprecipitated with Nr-CAM. This implies that Nr-CAM forms these molecular complexes by mechanisms that are RGD-independent. In agreement with this view, L1 forms complexes with integrins by both RGD-dependent and -independent mechanisms (19, 22, 34). Hence, in addition to its role in homophilic cell-cell adhesions, the shed Nr-CAM may act by both autocrine mechanisms and in a paracrine fashion by binding to integrins expressed on the surface of the same, or neighboring cells (Fig. 7).

**Nr-CAM and tumorigenesis.** During tumor development and metastasis, infiltration of endothelial cells into the tumor mass, induction of blood vessel formation, and invasion of tumor cells into blood vessels, all involve an intimate crosstalk between tumor and endothelial cells and lead to coordinated migration of these cells. Studies on molecules regulating neuronal guidance and migration suggest that “extracellular molecules” including netrins, semaphorins, ephrins, and Slits, also have a function in tumor angiogenesis and metastasis (35, 36). DNA microarray analysis of genes induced during the organization of endothelial cells into blood vessels showed that Nr-CAM, similar to some of these molecules, is induced during the morphogenetic changes involved in this process (37, 38). Therefore, Nr-CAM may function in attracting endothelial cells and stimulating the growth of blood vessels in the tumor proximity, thereby promoting tumor cell growth.

Together, these studies imply that molecules involved in the regulation of neuronal cell migration also control more general mechanisms that enhance cell motility. In addition, the induction of Nr-CAM in tumor cells by hyperactive β-catenin (4) could represent another case of opportunistic activation of target genes that normally regulate cell motility in other tissues and which, in cancer cells, results in enhanced proliferation, motility, and vascularization of the tumor.

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