# Cell Adhesion Molecule L1 Disrupts E-Cadherin-Containing Adherens Junctions and Increases Scattering and Motility of MCF7 Breast Carcinoma Cells

**Michael Shtutman, Elina Levina, Patrice Ohouo, Mirza Baig, and Igor B. Roninson**

Cancer Center, Ordway Research Institute, Albany, New York

## Abstract

**The first steps of invasion and metastasis include the dissociation of adherens junctions and the induction of migratory phenotype, through a program that resembles epithelial-mesenchymal transition (EMT). The L1 cell adhesion molecule, which is normally found primarily in the brain, was recently shown to be expressed in different types of cancer and to have tumor-promoting activity. We now find that L1 mediates EMT-like events in MCF7 breast carcinoma cells. MCF7 predominantly expresses the nonneuronal isoform of L1, as do 16 of 17 other cell lines derived from different types of cancer. L1 protein expression in MCF7 cells, which form E-cadherin-containing adherens junctions, is inversely related to cell density. Analysis of MCF7 cells with overexpression or knockdown of nonneuronal L1 isoform revealed that L1 expression leads to the disruption of adherens junctions and increases β-catenin transcriptional activity. As a result, L1 expression promotes the scattering of epithelial cells from compact colonies. Expression of the full-length L1 protein, but not of its soluble extracellular moiety, increases the motility of the MCF7 epithelial monolayer in a wound-healing assay, in which L1 expression is preferentially observed and required in cells leading the movement of the monolayer. Based on these results, we propose a model for the role of L1 as a trigger of EMT-like events in transformed epithelial cells.** (Cancer Res 2006; 66(23): 11370-80)

## Introduction

Epithelial-mesenchymal transition (EMT) is the process by which polarized epithelial cells are converted into individually motile cells. A similar series of events takes place during tumor progression, when carcinoma cells stably or transiently lose epithelial polarities and acquire a mesenchymal phenotype. The important marks of EMT are the dissociation of adherens junctions and the induction of migratory phenotype, allowing cells to escape the surrounding epithelium and invade other tissues (1). Although several signal-transduction pathways have been recently identified for EMT (2), the processes that trigger EMT are poorly understood. The search for molecules triggering and promoting EMT may yield new targets to block invasion and metastasis.

L1 glycoprotein is a cell adhesion molecule that, until recently, was believed to be specific for the nervous system (3), and has been implicated in a number of neurologic disorders (4), although it was also found in the blood cells (5) and kidney (6). L1 comprises six immunoglobulin-like domains and five fibronectin type III repeats in the extracellular region, a single-pass transmembrane domain, and a cytoplasmic tail (Fig. 1*A*). Through its extracellular domains, L1 can interact with a variety of ligands (7). Tissue-specific differential splicing leading to skipping of mini-exons 2 and 27 in L1 (Fig. 1*A*) has been observed in tissues other than the brain (8, 9). L1 is a transmembrane molecule that can be cleaved by metalloproteinases and released as the soluble form (10).

It has been recently found that L1 is expressed in human tumors and that its expression is correlated with tumor progression. L1 has been found in a number of neuroendocrine tumors, including malignant melanomas, gliomas and schwannomas, small cell lung carcinomas, and pancreatic neuroendocrine carcinomas (11–15). Recently, it has been shown that levels of L1 were correlated with poor prognosis, invasion, and metastasis of many epithelial tumors: L1 is expressed at the invasive front of colon cancer (16) and its expression correlates with metastasis of clear cell renal carcinomas (17). In ovarian and uterine epithelial tumors, L1 expression correlates with poor prognosis and reduced survival rate; it shows specific localization at the advancing edge of tumors during their adjustment to stroma (18). The role of L1 in cancer has been indicated not only by its expression pattern but also by functional analysis. L1 was identified by function-based transcriptome-scale screening as a molecule that affects the growth of breast carcinoma cells (19). L1 overexpression was subsequently found to transform NIH 3T3 cells, and its expression was shown to promote the motility, invasion, and tumorigenesis of colon carcinoma cells (16). Despite this evidence, the role of L1 in tumor progression is poorly understood at the mechanistic level.

Here, we show that L1 plays a role in EMT-like events in MCF7 breast carcinoma cells. We find that L1 inhibits cadherin-based adherens junctions formation, stimulates β-catenin signaling, and promotes the motility of epithelial cells, serving as a functional determinant of the cells that lead the movement of the epithelial monolayer. Our results suggest that L1 is a molecule that triggers EMT, providing a previously unknown molecular mechanism of EMT regulation and justifying L1 as a new mechanistic target for antimetastatic therapy.

## Materials and Methods

**Cell culture conditions.** Most of the cell lines were obtained from American Type Culture Collection (Manassas, VA), except for U251 glioblastoma (from Dr. O. Volpert, Northwestern University, Chicago, IL) and A2780 ovarian carcinoma (from Dr. S. Howell, University of California, San Diego, CA). MCF7 cells and their derivatives were maintained in DMEM supplemented with 0.1 mmol/L of nonessential amino acids,

**Note:** Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

M. Shtutman and E. Levina contributed equally to this work.

**Requests for reprints:** Michael Shtutman, Ordway Research Institute, 150 New Scotland Avenue, Albany, NY 12208. Phone: 518-641-6484; Fax: 518-641-6305; E-mail: mshtutman@ordwayresearch.org.



doi:10.1158/0008-5472.CAN-06-2106

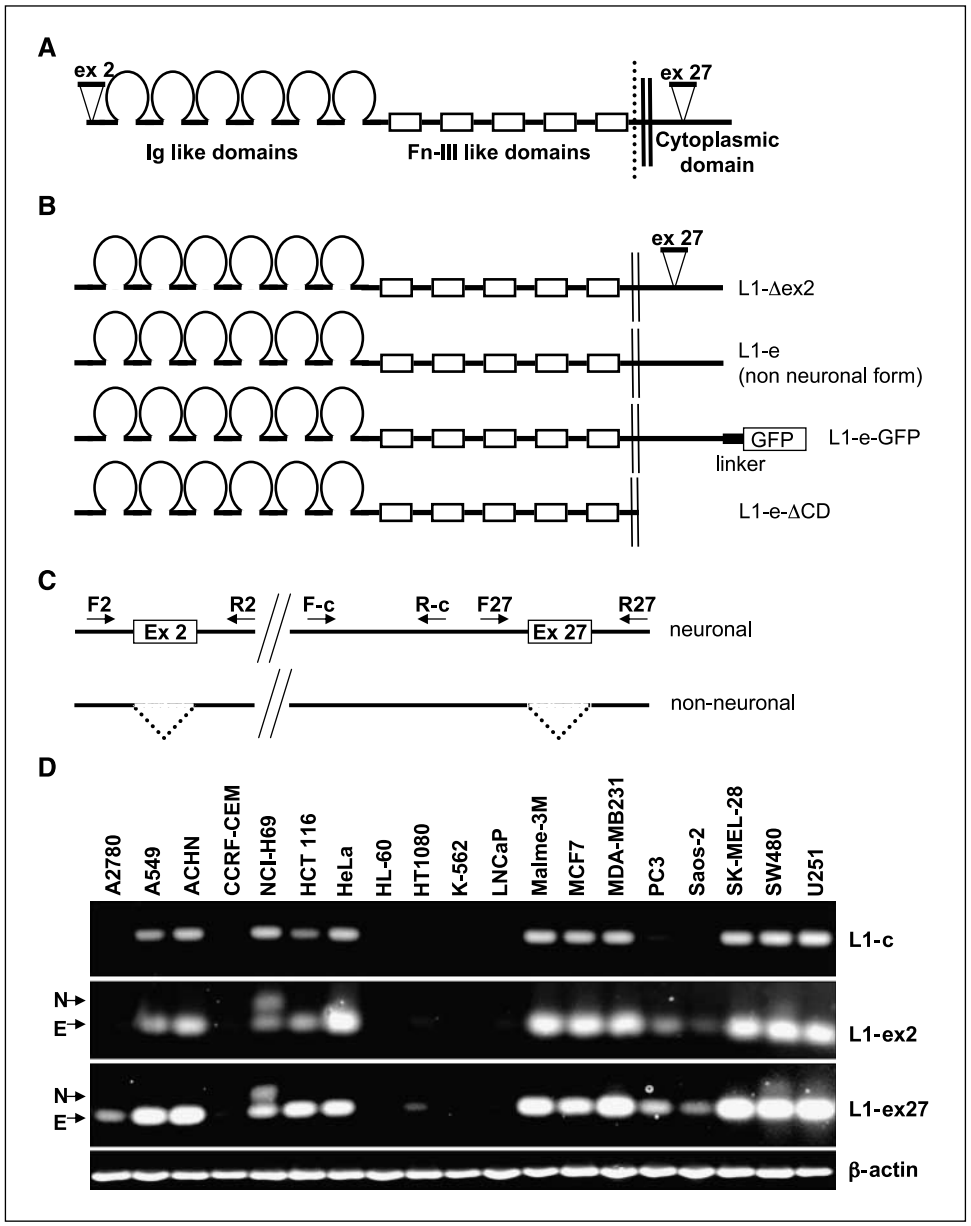


**Figure 1.** RT-PCR analysis of L1 splice variants in tumor cell lines. *A,* domain structure of L1. The positions of neuronal isoform-specific exons 2 and 27 that are spliced out in the nonneuronal isoform are marked. The transmembrane domain (*two vertical lines*), and the site of metalloproteinase cleavage that generates soluble L1 (*dotted line*) are marked. *B,* scheme of L1 derivatives used in this study (see Materials and Methods). *C,* scheme of PCR primer pairs. F-c/R-c primers amplify a 130 bp fragment common to both isoforms of L1 (L1-c). F-2/R-2 primers amplify a 128 bp product specific for the neuronal isoform and a 113 bp product for the nonneuronal isoform (L1-ex2). F-27/R-27 primers amplify 108 and 96 bp fragments specific for the neuronal and nonneuronal isoforms, respectively (L1-ex27). *D,* DNA electrophoresis of PCR-amplified fragments of L1. RNA from the following cell lines were used as a template. Carcinomas: lung A549, NCI-H69; colon HCT116, SW480; breast MCF7, MDA-MB231; prostate PC3, LNCaP; cervical HeLa; ovarian A2780; renal ACHN. Sarcomas: fibrosarcoma HT1080, osteosarcoma Saos-2. Leukemias: K562, HL60, CCRF-CEM. Melanoma: Malme-3M, SK-MEL-28. Glioblastoma: U251. *Arrows,* neuronal (*N*) and nonneuronal (*E*) specific PCR products.

1 mmol/L of sodium pyruvate, 0.01 mg/mL of bovine insulin (Sigma, St. Louis, MO), and 10% fetal bovine serum (FBS; HyClone). MDA-MB231, HeLa, SW480, HCT116, HT1080, ACHN, K562, SK-MEL-28, and U251 cell lines were grown in DMEM with 10% FBS. HL60 cells were grown in DMEM with 20% FBS. CCRF-CEM, LNCaP, PC3, NCI-H69, and A2780 cell lines were grown in RPMI with 10% FBS. A549 and Malme-3M cells were grown in Ham's F12 and L-15 medium, correspondently with 10% FBS. Saos-2 cells were grown in McCoy's 5A with 20% FBS.

**Retroviral infection.** Pantropic retroviral transduction was done as described (20) using pCL-eco (ref. 21; Imgenex, San Diego, CA) and pVSV-G packaging constructs (Clontech, Mountain View, CA). Vector plasmid, pCL-eco, and pVSV-G DNA were mixed at a 5:3:2 ratio and cotransfected using calcium phosphate protocol into 293T cells. Retrovirus-containing supernatants were harvested twice, at 24 and 48 hours after transfection. Cells were infected using a centrifugation-infection procedure (22), with 8 μg/mL of polybrene and infected cell populations were selected either with G418 or by fluorescence-activated cell sorting (FACS) for green fluorescent protein (GFP) fluorescence. The results of post-sorting FACS analysis are shown in Supplemental Fig. S1.

**Promoter assays.** β-Catenin-dependent promoter activity was analyzed by transient transfection of pTOPFLASH and pFOPFLASH plasmids (23), kindly provided by Dr. H. Clevers (Hubrecht Laboratorium, NIOB/KNAW, Center for Biomedical Genetics, Utrecht, the Netherlands). Confluent MCF7 cells were transfected with a mixture of 0.05 μg of pCMV-renilla normalization vector expressing renilla luciferase, 0.01 μg of TOPFLASH or FOPFLASH reporter vectors expressing firefly luciferase, and 2 μg of pCDNA-3 vectors expressing L1 (pCDNA3-L1-e) or GFP (pCDNA3-GFP), using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). Luciferase activities were measured using Dual-Glo luciferase assay system (Promega, Madison, WI) according to the manufacturer's protocol.

**Plasmids and vectors.** Three L1-targeting short hairpin RNAs (shRNA) were generated and inserted into pLPCX-H1RNA-GFP retroviral vectors (to be described elsewhere) and tested by immunoblotting for L1 knockdown. Of these, shRNA-L1-3 (GATCCCCAGACCAGAAGTACCGGATATTCAAGAGAAATCCGGTACTTCTGGTCTTTTTTGGAAA and AGCTTTTCCAAAAAAGACCAGAAGTACCGGATTTCTCTTGAATATCCGGTACTTCTGGTCTGGG) showed the best activity and was used in subsequent experiments, whereas shRNA-L1-1 (GATCCCCGAAAGGTTCCAGGGT-

GACCTTCAAGAGAGGTCACCCTGGAACCTTTCTTTTTGGAAA and AGCTTTTCCAAAAAGAAAGGTTCCAGGGTGACCTCTCTTGAAGGTCACCCTGGAACCTTTCGGG) was found to be totally inactive and used as a negative control.

Vectors expressing different forms of L1 protein were generated from pCDNA3-L1 vector expressing the neuronal isoform of human L1 (ref. 24; a gift from Dr. V. Lemmon, The Miami Project to Cure Paralysis, University of Miami, Miami, FL). To obtain the shRNA-resistant variant of L1 cDNA (pCDNA3-L1r), a silent mutation was introduced into the shRNA binding site using QuikChange II-E site-directed mutagenesis kit (Stratagene, La Jolla, CA) with the following oligonucleotides (sense, GCTGGCCAAAGATCAAAAATACCGCATTCAGC; antisense, GCTGAATGCGGTATTTTTGATCTTTGGCCAGC). To generate vectors expressing the nonneuronal isoform of L1, the neuronal-specific exons 2 and 27 were deleted using QuikChange II-E kit. At first, exon 2 was removed using sense TTATCCAGATCCCCGAGGAATTGATGGAGCCACCTGTCATC and antisense GATGACAGGTGGCTCCATCAATTCCTCGGGGATCTGGATAA primers, to provide pCDNA3-L1-Δex2. Then exon 27 was removed using sense GATGAGACCTTCGGCGAGTACAGTGACAACGAGGAGAAGGC and antisense GCCTTCTCCTCGTTGTCACTGTACTCGCCGAAGGTCTCATC primers, to provide nonneuronal L1 isoform (pCDNA3-L1-e).

For construction of L1-GFP fusion protein, the COOH-terminal part of L1 was amplified using sense primer ACAGCGGGTGAAAACTACAGTGTCGTCTCCTG and antisense primer ATTAGCGGCCGCTTCCGCCGGTTCCCCCGCCACTACCCCCTTCTAGGGCCACGGCAGGG, containing the sequence encoding GGSGGGTGGSG linker, that was previously used for the construction of L1-CFP fusion protein (25). Amplified fragments were inserted into pCDNA3-L1-e vector using *Bsm*BI and *Not*I sites. PCR-amplified enhanced green fluorescent protein was then inserted in frame with L1 into the *Not*I and *Xho*I sites of the resulting construct, to provide pCDNA3-L1-GFP.

To provide the L1 variant with deleted cytoplasmic domain, the COOH-terminal part of L1 extracellular and transmembrane domains was amplified using sense primer ACAGCGGGTGAAAACTACAGTGTCGTCTCCTG and antisense primer ATTCCTCGAGCTAGATGAAGCAGAGGATGAGCAG, containing a stop codon before the *Xho*I site. The resulting product was inserted into pCDNA-L1-e vector using *Bsm*BI and *Xho*I sites instead of the full-length COOH-terminal part of L1.

The sequences of all the obtained constructs were verified by DNA sequencing. The scheme of the constructs is depicted in Fig. 1*B*. To construct L1-expressing retroviruses, all the constructed L1 variants were inserted into a modified pLNCX retroviral vector using *Hind*III and *Xho*I sites. The retroviral vector expressing the p120 catenin–targeting shRNA has been described (26).[1]

**PCR assays.** To analyze the expression of L1 in tumor cell lines, total cellular RNA was isolated using RNeasy Mini Kit (Qiagen, Valencia, CA) and treated with RNase-free DNase I (Qiagen). cDNAs were synthesized using SuperScript III first-strand synthesis system for reverse transcription-PCR (RT-PCR; Invitrogen, Carlsbad, CA) priming with random hexamers. L1 fragments were amplified by PCR using Klentaq DNA polymerase (Sigma). The L1 fragment common to both neuronal and nonneuronal isoforms was amplified using F-c GACTACGAGATCCACTTGTTTAAGGA and R-c CTCACAAAGCCGATGAACCA primers. The exon 2–containing segment was amplified using F-2 CTGCCTGCTTATCCAGATCC and R-2 CCTCACACTTGAGGCTGATG primers to provide 128 and 113 bp PCR products for neuronal and nonneuronal isoforms, respectively. The exon 27–containing area was amplified using F-27 GGCCCGACCGATGAAAG and R-27 TTGATGTCCCCGTTGAGC primers to provide 108 and 96 bp PCR products for neuronal and nonneuronal isoforms, respectively. β-Actin was amplified using bAC-f GCTCCTCCTGAGCGCAAG and bAC-r CATCTGCTGGAAGGTGGACA primers. Semiquantitative PCR of L1 in sparse and dense cultures of MCF7 cells was done using L1c-r and L1c-f primers; β-actin was used for normalization.

Quantitative PCR was done by the SYBR Green method using ABI 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). The reactions were set in a 96-well plate by mixing 2× concentrated SYBR Green Master Mix (Applied Biosystems) with the corresponding primers and cDNA samples, according to the manufacturer's protocol. L1 expression was analyzed using L1c-r and L1c-f primers. β-Actin, L32 ribosomal protein, glyceraldehyde-3-phosphate dehydrogenase, and Ubch5B were used for normalization as described (27).

**Protein measurement.** Cells were plated at an equal density and one plate from each arm of the experiment was used to count cells prior to protein extraction, to assure equal protein concentrations. Protein levels were analyzed by immunoblotting using the enhanced chemiluminescence method (Amersham Biosciences), with the following primary antibodies: mouse anti-L1-CAM UJ127 (NeoMarkers), mouse anti-α-tubulin DM1A (Sigma), mouse anti-E-cadherin (Zymed), mouse anti-p120ctn (BD Bioscience PharMingen, San Diego, CA), and mouse anti-GFP (Roche). Fractionation of proteins into Triton X-100–soluble and -insoluble fractions was done as previously described (28), and equal volumes of lysates from the two fractions were analyzed. X-ray densitometry was carried out using VersaDoc Model 4000 Imaging System (Bio-Rad, Hercules, CA). Images were quantified using Quantity One one-dimensional analysis software. The brightness, contrast, and final size of the images were adjusted using Adobe Photoshop CS.

**Immunofluorescence.** Cells cultured on glass coverslips (Bellco Glass, Vineland, NJ) were fixed with 3.7% paraformaldehyde in PBS for 20 minutes at room temperature and permeabilized with 0.5% Triton X-100 in PBS for 3 minutes. The coverslips were incubated with mouse anti-L1 5G3 (BD Bioscience PharMingen), or mouse anti-E-cadherin (Zymed) antibodies, or TRITC-Phalloidin (Sigma). The secondary antibodies were Alexa Fluor 488- or Alexa Fluor 594–conjugated goat anti-mouse IgG (Molecular Probes, Eugene, OR). Fluorescence images were acquired using Leica DM IRE2 microscope equipped with Leica cooled CCD camera, Leica FW4000 software and HCX PL Fluotar L 40×/0.6 NA, HCX PL Apo 63×/1.4 NA, or HCX PL Apo 100×/1.4 NA objectives. The brightness, contrast, color balance, and final size of the images were adjusted using Adobe Photoshop CS.

**Colony scattering assay.** One thousand cells were plated per 100 mm plate and allowed to form small colonies for 5 days. One hundred phase-contrast images of fixed colonies from each cell line were analyzed, and the number of cells maintaining contact with their neighbors was counted for each colony (Supplemental Fig. S2). The experiments were done thrice independently.

**Wound healing assays.** Confluent cell cultures were grown on 25 mm square glass coverslips (Corning) for 48 hours. Wounds were made with the tip of a micropipette or by removing half of the monolayer with a sterile razor blade. Cells were maintained in modified Rose chambers (29) in Leibovitz's L-15 medium without phenol red (Invitrogen) with 10% FBS and antibiotics. To analyze cell motility, phase contrast time-lapse microscopy was done in a 37°C room using Axiovert 200M (Zeiss) microscope equipped with a 10×/0.25 NA CP-Achromat objective and AxioCam MR camera. Images were collected every 5 minutes for 18 hours. To analyze "leader" cell distribution, cells were fixed 24 hours after wound formation and analyzed using phase contrast microscopy with a 10×/0.25 NA CP-Achromat objective. Seven hundred cells at each wound edge were scored; cells with large lamellipodia at the top of the monolayer outgrowth were counted as leader cells (30).

**Live cell multimode time-lapse imaging.** Near-simultaneous GFP fluorescence/phase contrast time-lapse sequences were collected on a Leica ASMDW Imaging system equipped with Roeper HQ cooled camera and xenon monochromator using 40×/0.55 NA long working distance objective. The workstation was located in a temperature-controlled box stabilized at 37°C. Images were collected every 3 minutes for 18 hours using Leica ASMDW software.

## Results

**Most tumor cell lines express predominantly nonneuronal L1 isoforms.** Because L1 can be expressed as either the

[1] Boguslavsky et al., in preparation.

neuronal or the nonneuronal splicing isoforms, and because neuronal-specific exons 2 and 27 contain binding sites for L1-interacting proteins and are important for L1-potentiated cell migration (31–33), we were interested to determine which isoforms were expressed in different types of cancer. For this analysis, we used RT-PCR to analyze L1 expression in a panel of tumor cell lines including lymphomas, sarcomas, carcinomas, and tumors of neuroendocrine origin (Fig. 1*D*). In these assays, L1-c represents a PCR product common for both isoforms, and L1-ex2 and L1-ex27 are PCR products that include neuronal-specific exons 2 and 27, respectively (Fig. 1*C*), producing different-size fragments for the neuronal and the nonneuronal isoforms. As shown in Fig. 1*D*, a great majority of cell lines were positive for L1, but only one line (NCI-H69 small cell lung carcinoma) expressed both isoforms in equal amounts. All the other lines predominantly expressed the nonneuronal isoform, including the two tested breast carcinoma cell lines, MCF7 and MDA-MB231.

**Density-dependent expression of L1 in breast carcinoma cells.** Immunofluorescence staining of L1 in MCF7 cells showed a striking difference among cell cultures of different density. As shown in Fig. 2*A*, a sparse culture of MCF7 cells shows L1 localization in the cytoplasm. In a semiconfluent culture (medium density), L1 was redistributed to the cell periphery at the cell-cell contact sites. At full confluence (dense culture) L1 staining was practically undetectable (Fig. 2*A*). The dramatic reduction of L1 protein levels in dense culture was confirmed by immunoblotting (Fig. 2*B*). RT-PCR analysis (Fig. 2*C*) indicated that cell density regulates L1 expression at the mRNA level, and quantitative PCR analysis showed that dense cultures contained ~5-fold less L1 mRNA than sparse cells. Transcriptional regulation was confirmed by comparing the effect of cell density on the expression of endogenous L1 and of exogenous L1-GFP fusion protein transduced into MCF7 cells. In dense cultures, the levels of endogenous protein decreased, but the levels of exogenous L1-GFP were unchanged (Fig. 2*D*).

An important step during the transition from the sparse to the dense epithelial culture is the formation of E-cadherin-positive adherens junctions. Immunofluorescence staining of MCF7 cells showed typical E-cadherin localization at the sites of cell-cell junctions (Fig. 2*A*).

In contrast to MCF7, MDA-MB231 breast carcinoma cells do not form developed adherens junctions and do not express cadherins, except for mesenchymal cadherin 11 and its recently identified homologue, cadherin 24 (34–36). Whereas epithelial cadherins such as E-cadherin are responsible for the formation and maintenance of epithelial structures, expression of cadherin 11 correlates with the migratory phenotype (37). Immunofluorescence staining and immunoblotting showed that localization and expression of L1 was similar in sparse and dense cultures of MDA-MB231 cells (Fig. 2*E* and *F*), suggesting that down-regulation of L1 in dense cultures may require the formation of E-cadherin-based adherens junctions.

To test the role of adherens junctions in density dependence of L1 expression, we inhibited adherens junction formation in MCF7 cells by a knockdown of p120 catenin, the key regulator of cell-cell adhesion (38). MCF7 cells with p120ctn knockdown were obtained by infection with a retrovirus expressing p120ctn-targeting shRNA followed by puromycin selection. The infected cells show lower levels of p120ctn (Fig. 2*G*), and had impaired cadherin-mediated cell-cell junctions despite undiminished levels of adherens junction proteins, E-cadherin, β-catenin, and α-catenin.[1] L1 immunoblotting analysis showed that knockdown of p120ctn prevents the loss of L1 in dense cultures (Fig. 2*H*), indicating that E-cadherin-mediated adherens junctions are required for density-dependent regulation of L1 in MCF7 cells.

**L1 overexpression decreases the junctional level of E-cadherin and enhances β-catenin-dependent transcription.** To determine if L1 expression affects adherens junctions formation, we compared MCF7 cells expressing exogenous L1-GFP fusion protein, and control cells transduced with a control vector that expresses GFP alone (Fig. 3). The intensity of E-cadherin immunofluorescence was lower in cells overexpressing L1-GFP (Fig. 3*A*). Higher magnification showed that E-cadherin was sharply localized to cell-cell contacts in control cells, but cell-cell contacts became disorganized and more E-cadherin appeared in the cytoplasm of L1-GFP-overexpressing cells (Fig. 3*B*). E-cadherin exists in detergent-soluble and -insoluble fractions; when adherens junctions form, E-cadherin becomes stabilized at the cell cortex and is insoluble. We compared the amounts of E-cadherin in detergent-soluble and -insoluble fractions in FACS-purified populations of control and L1-GFP-expressing cells by immunoblotting (Fig. 3*C*). L1-GFP did not significantly change the total amount of E-cadherin but induced the redistribution of E-cadherin from the detergent-insoluble to the -soluble fraction (Fig. 3*C*). The ratio of soluble to insoluble E-cadherin band intensity was >3-fold higher in L1-GFP-expressing cells compared with the control ($P < 0.002$, *t* test; Fig. 3*D*).

The release of E-cadherin from adherens junctions leads to the disruption of the complex between E-cadherin and β-catenin. In tumor cells (where β-catenin degradation is impaired), this is followed by relocalization of β-catenin to the nucleus where it forms a complex with LEF/TCF DNA-binding proteins involved in transcription regulation (39). Increased nuclear staining for β-catenin was not observable by immunofluorescence microscopy analysis in L1-overexpressing cells (data not shown), but an increase in β-catenin activity was detected by a more sensitive transcription-transactivating assay. In this assay, we coexpressed L1 in MCF7 cells together with a reporter plasmid, pTOPFLASH, that contains a LEF/TCF binding site for β-catenin regulating the expression of luciferase reporter (23). L1 overexpression produced a moderate but statistically significant increase ($P < 0.005$; *t* test) in β-catenin-dependent promoter activity (Fig. 3*E*).

**L1 regulates colony scattering of MCF7 cells.** The above results show that overexpression of L1 disrupts the adherens junctions, causes the release of E-cadherin, and increases β-catenin-dependent transactivation. To investigate the role of L1 in epithelial cell adhesion, we developed derivatives of MCF7 cells with either overexpression of L1-GFP or knockdown of endogenous L1. L1 knockdown cells were prepared by infecting MCF7 with retroviruses expressing L1-targeting shRNA and GFP marker followed by FACS selection (Supplemental Fig. S1). Cells expressing inactive shRNAs were used as a control. Levels of L1 knockdown (~80%) or L1-GFP fusion protein expression were determined by immunoblotting (Fig. 4*A* and *B*).

MCF7 derivatives were analyzed by a colony scattering assay, which measures the ability of epithelial tumor cells to detach from colonies in culture, mimicking certain aspects of tumor invasion (40). Cells were plated at a very low density and the morphology of colonies was analyzed 5 days after plating. Colonies were divided into three categories: compact (in which

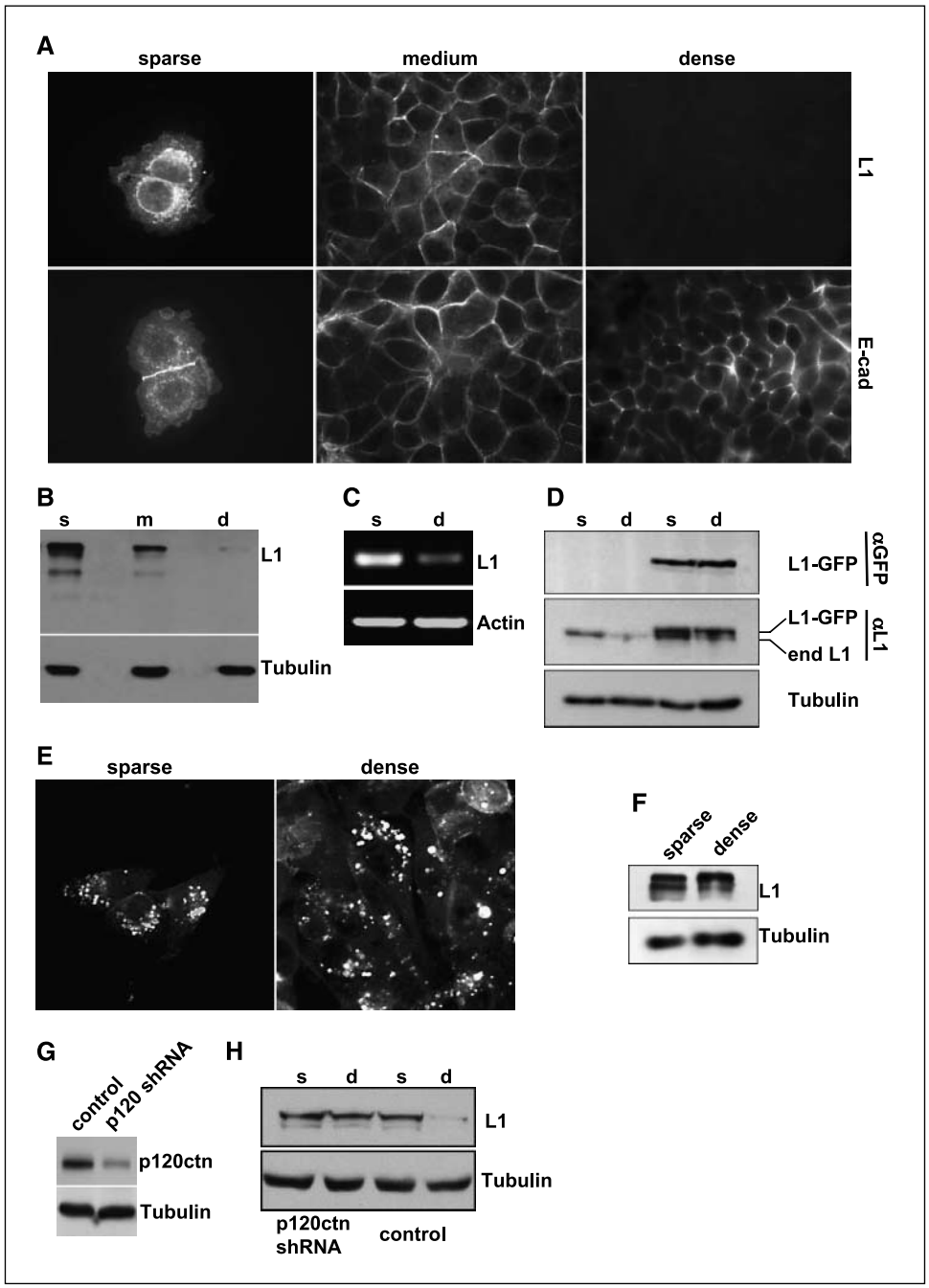


**Figure 2.** Cell density dependence of L1 expression in breast carcinoma cells. *A,* immunofluorescence staining of sparse, medium, and dense cultures of MCF7 cells with anti-L1 (*top*) and anti-E-cadherin (*bottom*) antibodies. *B,* immunoblotting of L1 in sparse (*s*), medium (*m*), and dense (*d*) cultures. *Bottom,* tubulin was used as a loading control. *C,* RNA levels of L1 in sparse and dense MCF7 cultures analyzed by RT-PCR. *Bottom,* RT-PCR for β-actin (normalization standard). *D,* immunoblotting analysis of the expression of the endogenous L1 and exogenous L1-GFP fusion protein in sparse and dense cultures, using anti-L1 and anti-GFP antibodies. *E,* cytoplasmic localization of L1 in MDA-MB231 breast carcinoma cells in sparse and dense culture. *F,* immunoblotting of L1 in sparse and dense cultures of MDA-MB231 cells. *G,* immunoblotting of p120 catenin in control and anti-p120ctn shRNA–expressing MCF7 cells. *H,* immunoblotting of L1 in sparse (*s*) and dense (*d*) cultures of anti-p120ctn shRNA–expressing and control MCF7 cells.

>90% of cells in a colony have cell-cell junctions), loose (in which 50–90% of cells form junctions), and scattered (in which <50% of cells form junctions; Fig. 4*D*; Supplemental Fig. S2). Compact colonies had E-cadherin and F-actin localized in the area of cell-cell contacts. In cells detached from scattered colonies, E-cadherin stained diffusely in the cytoplasm, and F-actin was localized in the small lamellipodia at the cell edge (Fig. 4*E*).

L1-knockdown cells formed, on average, two times more compact colonies and four times fewer scattered colonies than L1-GFP-overexpressing cells. Control cells expressing inactive shRNA produced intermediate results (Fig. 4*C*). To confirm the target specificity of the inhibition of colony scattering by L1 shRNA, we expressed L1 cDNA resistant to L1 shRNA (see Materials and Methods) in L1-knockdown cells. The number of scattered colonies in these cells was similar to L1-GFP-expressing cells (data not shown). Hence, L1 promotes colony scattering of MCF7 cells.

**Regulation of cell motility by full-length but not by the soluble L1.** It has been previously shown that overexpression of L1 stimulates the motility of neurons, melanomas, and several other cell lines (10, 16, 41, 42). To determine whether L1 affects cell migration in breast carcinoma cells, we studied the effect of L1 expression on the motility of MCF7 cells in a monolayer by an *in vitro* wound healing assay (see Materials and Methods). L1 knockdown significantly inhibited the speed of wound closure by the monolayer of MCF7 cells (Fig. 5*A, B,* and *E*). To verify the

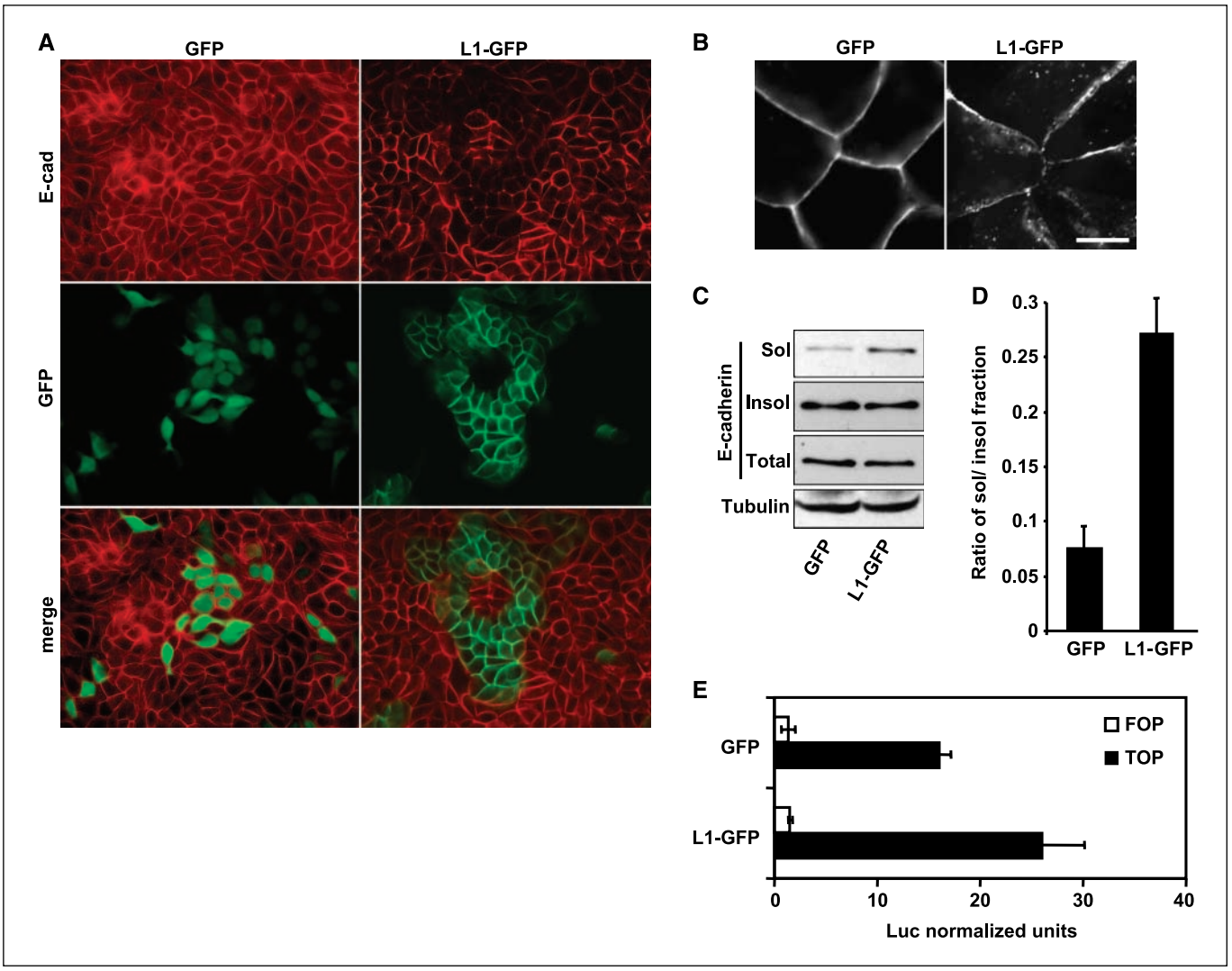


**Figure 3.** Effects of L1 overexpression on E-cadherin localization and β-catenin activity. *A,* E-cadherin localization in a dense culture of MCF7 cells expressing GFP (*left*) or L1-GFP fusion protein (*right*) cocultured with parental MCF7 cells. *B,* higher magnification images of E-cadherin staining in control GFP-expressing and L1-GFP-expressing MCF7 cells. *Bar,* 10 μm. *C,* total cell lysates or Triton X-100-soluble and -insoluble fractions from control GFP-expressing or L1-GFP-expressing MCF7 cells (96% pure by FACS) were analyzed by immunoblotting with anti-E-cadherin antibodies. *Bottom,* tubulin was used as a loading control. *D,* ratio of E-cadherin band intensity in soluble and insoluble fractions. *Columns,* mean of three independent experiments; *bars,* SD. *E,* firefly luciferase expression from reporter plasmids pTOPFLASH, containing trimeric β-catenin-responsive elements (Lef-1/TCF binding sites), and pFOPFLASH, containing mutated Lef-1/TCF binding sites (negative control). Reporter plasmids were cotransfected with vectors expressing L1-GFP or GFP (negative control), as well as pCMV-renilla expressing renilla luciferase (normalization control). The assays were carried out in triplicate. *Columns,* mean for firefly luciferase activity normalized to renilla luciferase; *bars,* SD.

specificity of the effect of shRNA, we expressed shRNA-resistant L1 in L1-knockdown cells (Fig. 5*E*). The restoration of L1 expression completely restored cell motility (Fig. 5*E*); both neuronal and nonneuronal isoforms of L1 were equally active in this assay (data not shown).

Regulation of motility by L1 has been previously attributed to the formation of soluble L1 fragments produced by the proteolytic cleavage of the extracellular domain of L1 (Fig. 1*A*) and its release into the culture media (10). To investigate the effect of the soluble form of L1 on the motility of MCF7 cells, we generated L1 knockdown cells expressing shRNA-resistant L1 deletion mutant lacking the cytoplasmic domain, L1-ΔCD (Fig. 5*F*). This mutant produces the same soluble L1 fragment as full-length L1 (Fig. 1*A*), but secretes it into the medium at a much higher level (Fig. 5*G*; ref. 43). Surprisingly, the motility of the knockdown cells was not restored by L1-ΔCD mutant (Fig. 5*E*), indicating that the effect of L1 on the motility of MCF7 cells is regulated by full-length molecules and not by its cleaved extracellular domain.

**L1-expressing cells lead the migration of the monolayer.** It has been recently shown that L1 localizes at the invasive front of human tumors (16, 18), suggesting that cells expressing elevated levels of L1 may preferentially migrate to the tumor edge. Epithelial cell migration involves the formation of leader cells with highly active leader lamellae and "follower" cells along the sides (30). Remarkably, we found that the L1 expression in a moving monolayer is significantly elevated in the leader cells, in which L1 is localized mainly in the perinuclear area and at the leading edge (Fig. 5*C*), similar to L1 distribution in the sparse cultures of MCF7 cells (Fig. 2*A*). L1 knockdown drastically

reduced the number of leader cells at the moving front of MCF7 monolayer (Fig. 5*D*), from 28% in parental MCF7 cells to 11% in cells expressing L1 shRNA.

To verify that L1 expression regulates the leader phenotype, we did a wound healing assay using a 1:1 mixture of parental MCF7 and cells expressing L1 shRNA and GFP. In control experiments, we used a mixture of parental cells and cells expressing GFP alone. Immediately after wounding, we observed equal numbers of green (GFP-expressing) L1-knockdown cells and colorless parental cells at the wound edge. After 18 hours, the wound edge consisted entirely of colorless cells, whereas the L1-knockdown green cells were behind the monolayer. In the control mixture, the distribution of green and colorless cells was not changed during the experiment (Fig. 6*A–D*; Supplemental Movies 1 and 2). Thus, L1 expression promotes the ability of cells to lead the movement of the monolayer.

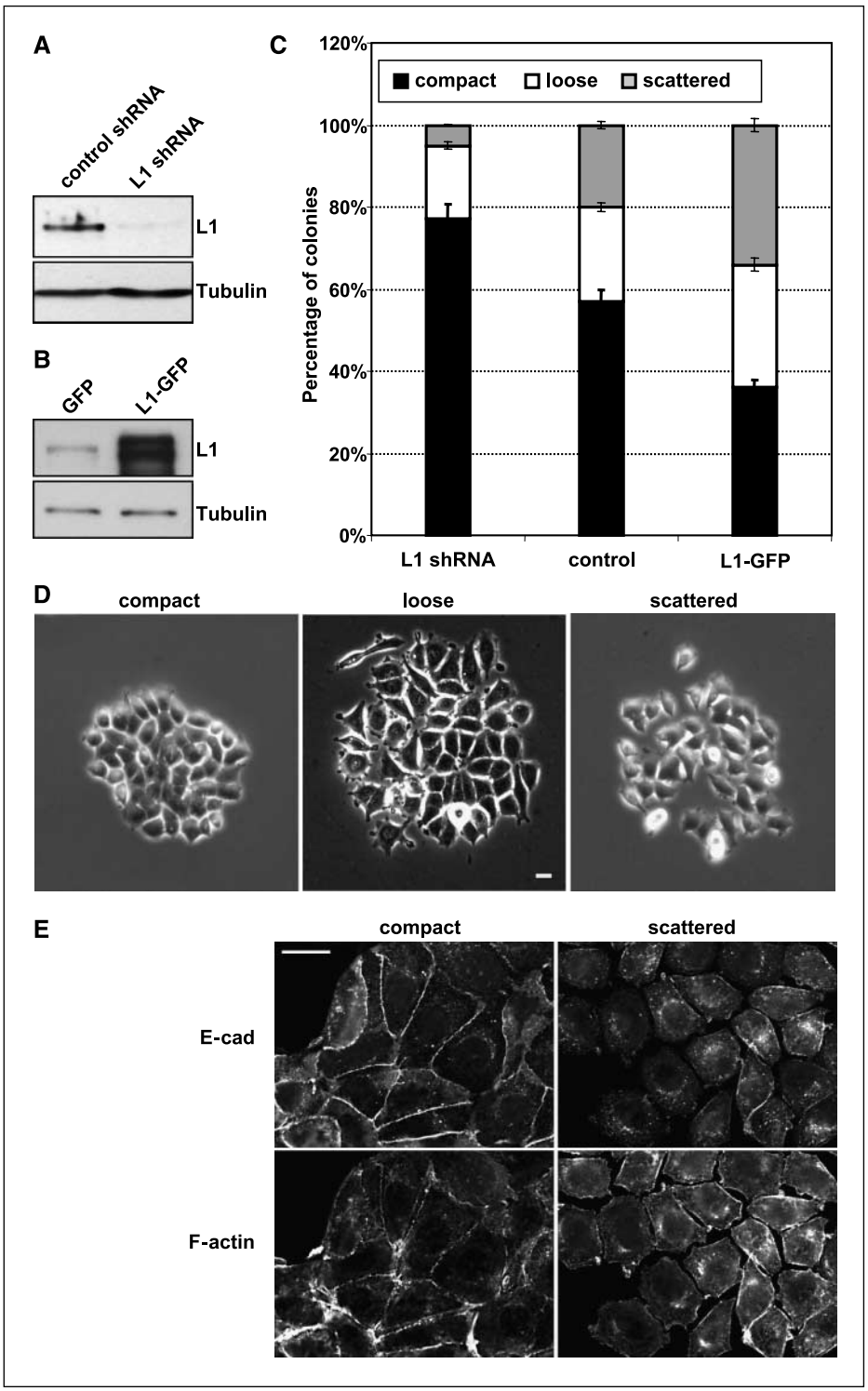


**Figure 4.** Effects of L1 on the scattering of MCF7 colonies. *A,* immunoblotting of L1 in MCF7 cells expressing control (inactive) shRNA or L1-specific shRNA. *B,* immunoblotting of L1 and L1-GFP fusion proteins in MCF7 cells infected with enhanced green fluorescent protein (GFP control) or L1-GFP-expressing retroviruses. *C,* distribution of compact, loose, and scattered colonies in L1 knockdown (*L1 shRNA*), inactive shRNA (*control*) and L1 overexpressing (*L1-GFP*) MCF7 cells. Cells were plated at colony-forming conditions. One hundred colonies in each sample were categorized after scoring phase contrast images into three categories: (*a*) compact (>90% of cells in the colony have cell-cell contacts), (*b*) loose (50–90% cell contacts), (*c*) scattered (<50% cell contacts). *Bars,* percentages of compact, loose, and scattered colonies in each culture. The experiments were repeated thrice. *D,* examples of compact, loose, and scattered colonies of MCF7 cells. *E,* E-cadherin and F-actin in the compact and scattered colonies, visualized by double staining with anti-E-cadherin antibodies and FITC-phalloidin. *Bar,* 20 μm.

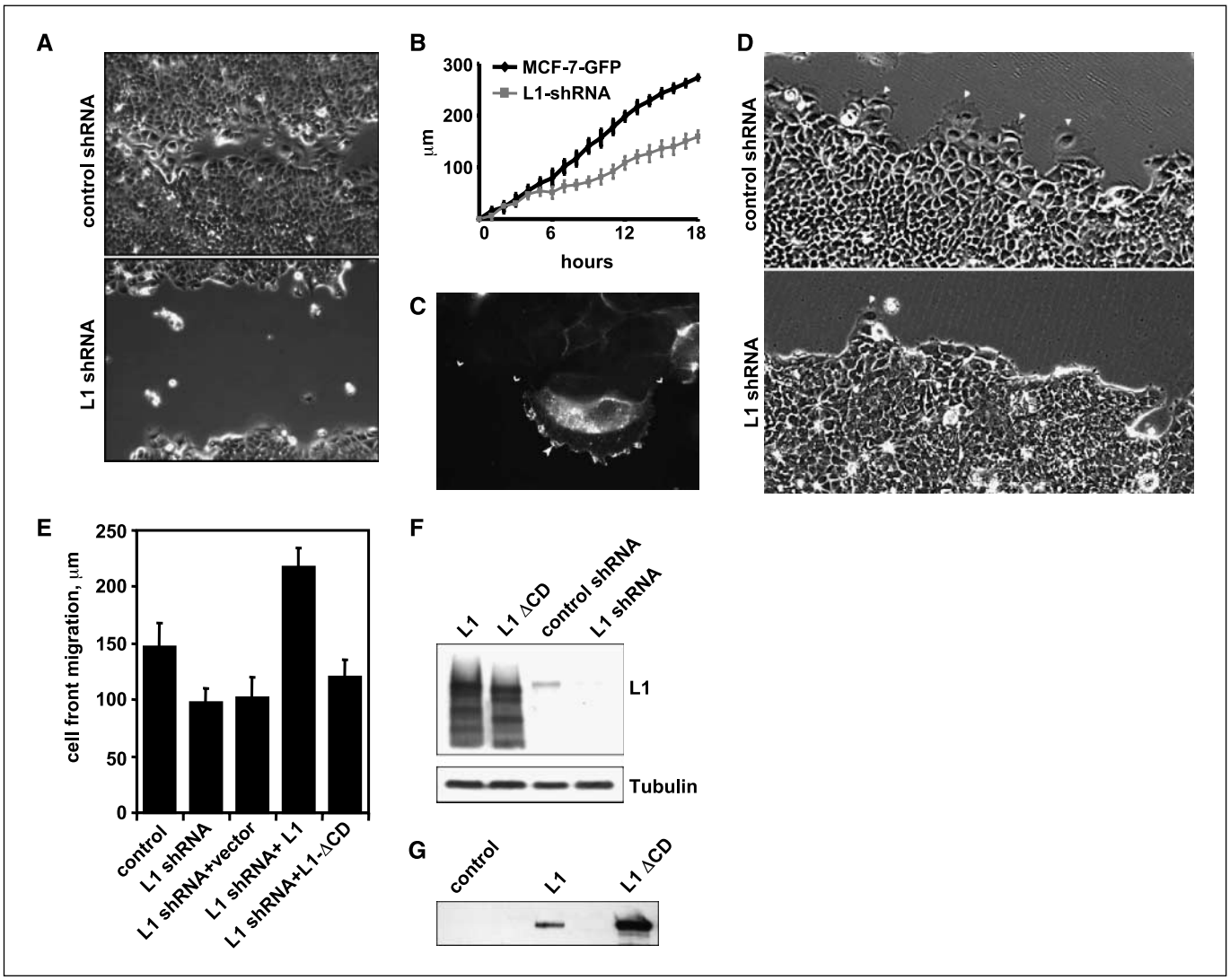


**Figure 5.** Effects of L1 on the motility of MCF7 cells. *A,* motility of MCF7 cells with control shRNA and with L1 knockdown in an *in vitro* wound healing assay. Images were taken 18 hours after wounding. *B,* rate of front migration of MCF7 cells monolayer with a control GFP-expressing vector and with L1 shRNA analyzed by time-lapse video microscopy. Images were taken every 5 minutes. The migration of the monolayer was measured every hour at six different points using Leica FW4000 software. *C,* staining with anti-L1 antibody shows elevated expression of L1 in an actively moving MCF7 leader cell at the wound edge (*large arrowhead*) relative to follower cells in the monolayer. *Small arrowheads,* wound edge and the direction of the movement. *D,* phase contrast images of the wound edge of MCF7 cells with control shRNA and with L1 knockdown, taken 18 hours after wounding. Leader cells are marked with arrowheads. *E,* monolayer front migration for 18 hours in the wound healing assay measured in cultures of MCF7 cells with control shRNA, L1 shRNA, and cells expressing L1 shRNA and transduced with an insert-free vector or shRNA-resistant full-length L1 or L1 lacking cytoplasmic domain (*L1ΔCD*). *F,* immunoblotting of L1 in MCF7 cells overexpressing full-length L1 (*L1*), L1 lacking cytoplasmic domain (*L1ΔCD*), control shRNA, or L1 shRNA. *G,* immunoblotting of soluble L1 in conditioned medium from similar amounts of control MCF7 cells or cells overexpressing either full-length L1 or L1ΔCD.

## Discussion

L1 cell adhesion molecule, originally believed to play a role only in the nervous system, was more recently found to be expressed in different types of cancer, to be a marker of more aggressive tumors, and to promote the growth, invasion, and motility of tumor cells (16, 19). The mechanism through which L1 affects tumor progression, however, has not been previously elucidated. In the present study, we have investigated the effects of the nonneuronal isoform of L1 in MCF7 breast carcinoma cells. Our results show that L1 promotes the release of E-cadherin from adherens junctions and activates β-catenin transcriptional activity. As a consequence, L1 expression disrupts the formation of adherens junctions, promotes scattering of colonies and stimulates the ability of epithelial cells to lead the movement of a monolayer. These findings provide the first demonstration that L1 promotes EMT-like transition in transformed epithelial cells, an event that plays a key role in tumor progression to metastatic competence.

We have found that expression of L1 mRNA and protein is negatively regulated by cell density in MCF7 but not in adherens junctions–negative MDA-MB231 breast carcinoma cells, suggesting that L1 expression may be regulated via E-cadherin/β-catenin signaling. The same density-dependent regulation of L1 was previously reported in colon carcinoma cells that formed E-cadherin-mediated cell-cell junctions (16). Inhibition of L1 expression by cell density is in agreement with very low levels of L1 expression in normal epithelial tissues. For example, L1 is found in the urinary tract, where it is expressed in the distal portions of the elongated tubules during development and then disappears

after the kidneys are formed (6). Such expression correlates with the patterns observed in our experiments, in which L1 is expressed in migrating cells and disappears after the formation of a dense epithelial monolayer. Higher expression of L1 in sparse cultures of colon carcinoma cells has been explained by positive regulation of L1 transcription by β-catenin, which is more active as a regulator of transcription in sparse cells where it is not bound to E-cadherin at adherens junctions (16, 39).

We have found that L1 expression causes the removal of E-cadherin from adherens junctions, resulting in increased β-catenin transcriptional activity. This disruption may be mediated by EGF receptor because L1 was shown to activate EGF-receptor (44), that in turn, activates β-catenin tyrosine phosphorylation, resulting in the disruption of E-cadherin/β-catenin interaction and translocation of β-catenin into the nucleus (45). Because β-catenin activation should further increase L1 transcription, our results suggest the existence of a positive feedback loop, which can lead to the rapid accumulation of L1 and the disappearance of adherens junctions.

Based on our results, we propose a model (Fig. 6*E*) describing L1 as a potential trigger of invasive behavior of carcinoma cells. Primary epithelial tumors (in a condition approximated by dense cultures) contain very low levels of L1. However, temporary elevation of the L1 levels due to the fluctuation of gene expression in individual cells (46) may trigger the activation of a positive feedback loop, where elevated levels of L1 decrease the level of E-cadherin at adherens junctions, resulting in the activation of β-catenin signaling and further accumulation of L1. As a result of the disruption of cell-cell contacts, L1-expressing cells become more motile and actively migrate to the tumor edge, where they detach from the tumor and invade the surrounding tissues.

Our findings of the effects of L1 on epithelial cell culture support the role for L1 in tumor invasion and can explain the observations of preferential L1 localization at the tumor edge of colon carcinomas (16, 39). In particular, we have found that L1 overexpression stimulates, and L1 knockdown inhibits, the scattering of compact colonies of epithelial cells, a property that was shown to correlate with tumor cell invasiveness (40, 47). We have also found that L1 overexpression increases and knockdown decreases the motility of MCF7 cell monolayer in wound healing assays. The motility-promoting effect of L1 was originally described in neurons (48), and was also shown in ovarian cell lines, CHO and SKOV3 (10). These cell lines are either cadherin-negative (CHO; ref. 49) or express a low level of E-cadherin and are unable to form developed adherens junctions (SKOV3; ref. 50). In all of these systems, the effects of L1 on the motility were found to be mediated by the soluble form of L1. In contrast, our data show that truncated L1, which lacks a cytoplasmic domain, overproduces soluble L1 but does not stimulate MCF7 cell migration, whereas the full-length protein has this activity. Furthermore, we have found that L1 is

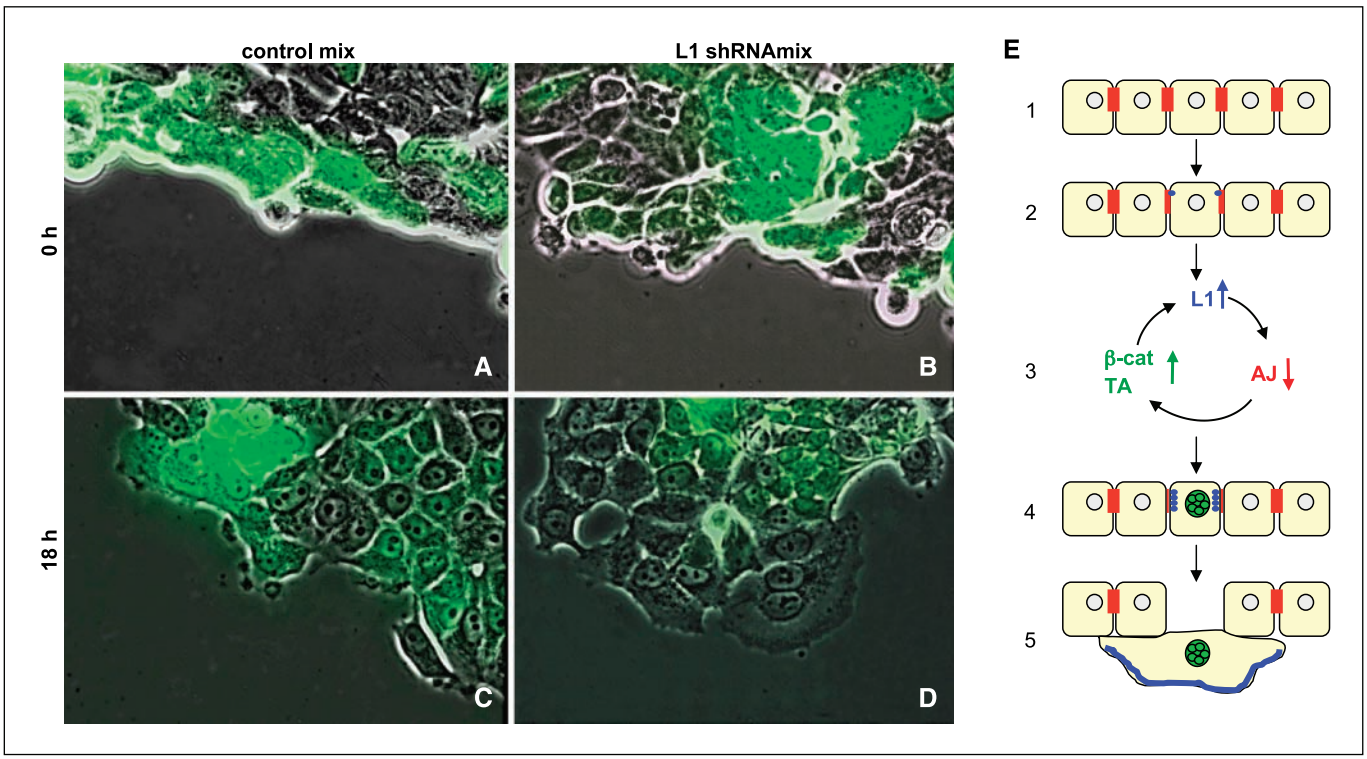


**Figure 6.** L1-expressing cells preferentially migrate into the wound edge. *A* to *D,* wound healing in a 1:1 mixture of colorless parental MCF7 cells and green L1-knockdown cells expressing GFP as a marker (*B* and *D*) shows that parental cells preferentially migrate into the wound. Mixture of parental MCF7 cells and cells expressing GFP alone was used as a control (*A* and *C*). First and last frames from 18-hour time-lapse movies are shown (see Supplemental Movies 1 and 2). *E,* model for initiation of invasion by L1 expression. (*1*) In differentiated carcinomas, E-cadherin-containing adherens junctions (*red lines*) are well developed and L1 is undetectable. (*2*) Stochastic fluctuations in gene expression lead to the appearance of L1 (*blue dots*) in some of the cells. (*3*) L1 expression leads to the disturbance of adherens junctions, removal of E-cadherin, and release of some β-catenin. The positive feedback loop is activated, wherein released β-catenin activates L1 transcription, leading to further reduction of adherens junctions and augmentation of β-catenin signaling. (*4*) L1 accumulation and β-catenin activation (*green dots* in the nucleus) leads to the disruption of adherens junctions. (*5*) Adherens junctions disruption is followed by cell detachment from the monolayer and invasion at the tumor edge.

preferentially expressed in those cells that lead the movement of the monolayer, and that L1 knockdown inhibits this leader phenotype. The requirement for L1 expression in leader cells is inconsistent with the role of soluble L1 as the determinant of cell migration, but is fully consistent with the role of cell-associated L1 as a regulator of cell-cell contacts. We hypothesize that full-length L1 regulates the migration of a cohesive monolayer of epithelial cells through its effects on signal transduction via the cytoplasmic domain, whereas cleaved soluble L1 may activate the motility of fibroblasts or more transformed cells lacking adherens junctions through its interaction with the surface of such cells. However, we also note that all the previous studies with soluble L1 were carried out using the neuronal isoform, the soluble derivative of which differs from the nonneuronal soluble isoform used here by five amino acids encoded by exon 2. Although exon 2 might be required for the effect of soluble L1 on cell motility, neither exon 2 nor exon 27 are essential for the activity of full-length L1 because both neuronal and nonneuronal full-length L1 isoforms are equally efficient in this assay.

The easy accessibility of L1 on the cell surface, its relative paucity in normal tissues (outside of the brain), and preferential L1 expression in more aggressive tumors make this protein a particularly appealing cancer drug target. In particular, anti-L1 monoclonal antibodies were shown to inhibit the growth of L1-expressing tumor cells both *in vitro* and *in vivo* (19, 51). Our identification of the mechanistic role of L1 as a trigger of invasion-promoting EMT-like transition lends further support to developing L1-targeting reagents for cancer therapy.

## Acknowledgments

Received 6/8/2006; revised 8/24/2006; accepted 10/4/2006.

**Grant support:** NIH grant R33 CA95996 (I.B. Roninson).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Drs. Vance Lemmon and Hans Clevers for the expression and reporter vectors; Natalie Warholic for excellent technical assistance and Dr. Lana Salov for help with various aspects of this study; and Drs. Mikhail Blagosklonny, Jacob Zhurinsky, Anna Kashina, and Wilfred Stein for helpful suggestions and critical reading of the manuscript.

## References

1. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2002;2:442–54.
2. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol 2006;7:131–42.
3. Salton SR, Richter-Landsberg C, Greene LA, Shelanski ML. Nerve growth factor-inducible large external (NILE) glycoprotein: studies of a central and peripheral neuronal marker. J Neurosci 1983;3:441–54.
4. Fransen E, Van Camp G, Vits L, Willems PJ. L1-associated diseases: clinical geneticists divide, molecular geneticists unite. Hum Mol Genet 1997;6:1625–32.
5. Ebeling O, Duczmal A, Aigner S, et al. L1 adhesion molecule on human lymphocytes and monocytes: expression and involvement in binding to αvβ3 integrin. Eur J Immunol 1996;26:2508–16.
6. Debiec H, Christensen EI, Ronco PM. The cell adhesion molecule L1 is developmentally regulated in the renal epithelium and is involved in kidney branching morphogenesis. J Cell Biol 1998;143:2067–79.
7. Haspel J, Grumet M. The L1CAM extracellular region: a multi-domain protein with modular and cooperative binding modes. Front Biosci 2003;8:s1210–25.
8. Coutelle O, Nyakatura G, Taudien S, et al. The neural cell adhesion molecule L1: genomic organisation and differential splicing is conserved between man and the pufferfish Fugu. Gene 1998;208:7–15.
9. Jouet M, Rosenthal A, Kenwrick S. Exon 2 of the gene for neural cell adhesion molecule L1 is alternatively spliced in B cells. Brain Res Mol Brain Res 1995;30:378–80.
10. Mechtersheimer S, Gutwein P, Agmon-Levin N, et al. Ectodomain shedding of L1 adhesion molecule promotes cell migration by autocrine binding to integrins. J Cell Biol 2001;155:661–73.
11. Fogel M, Mechtersheimer S, Huszar M, et al. L1 adhesion molecule (CD 171) in development and progression of human malignant melanoma. Cancer Lett 2003;189:237–47.
12. Hung G, Colton J, Fisher L, et al. Immunohistochemistry study of human vestibular nerve schwannoma differentiation. Glia 2002;38:363–70.
13. Kaifi JT, Zinnkann U, Yekebas EF, et al. L1 is a potential marker for poorly-differentiated pancreatic neuroendocrine carcinoma. World J Gastroenterol 2006;12:94–8.
14. Miyahara R, Tanaka F, Nakagawa T, et al. Expression of neural cell adhesion molecules (polysialylated form of neural cell adhesion molecule and L1-cell adhesion molecule) on resected small cell lung cancer specimens: in relation to proliferation state. J Surg Oncol 2001;77:49–54.
15. Tsuzuki T, Izumoto S, Ohnishi T, et al. Neural cell adhesion molecule L1 in gliomas: correlation with TGF-β and p53. J Clin Pathol 1998;51:13–7.
16. Gavert N, Conacci-Sorrell M, Gast D, et al. L1, a novel target of β-catenin signaling, transforms cells and is expressed at the invasive front of colon cancers. J Cell Biol 2005;168:633–42.
17. Allory Y, Matsuoka Y, Bazille C, Christensen EI, Ronco P, Debiec H. The L1 cell adhesion molecule is induced in renal cancer cells and correlates with metastasis in clear cell carcinomas. Clin Cancer Res 2005;11:1190–7.
18. Fogel M, Gutwein P, Mechtersheimer S, et al. L1 expression as a predictor of progression and survival in patients with uterine and ovarian carcinomas. Lancet 2003;362:869–75.
19. Primiano T, Baig M, Maliyekkel A, et al. Identification of potential anticancer drug targets through the selection of growth-inhibitory genetic suppressor elements. Cancer Cell 2003;4:41–53.
20. Yee JK, Friedmann T, Burns JC. Generation of high-titer pseudotyped retroviral vectors with very broad host range. Methods Cell Biol 1994;43 Pt A:99–112.
21. Naviaux RK, Costanzi E, Haas M, Verma IM. The pCL vector system: rapid production of helper-free, high-titer, recombinant retroviruses. J Virol 1996;70:5701–5.
22. Kotani H, Newton PB III, Zhang S, et al. Improved methods of retroviral vector transduction and production for gene therapy. Hum Gene Ther 1994;5:19–28.
23. van de Wetering M, Cavallo R, Dooijes D, et al. Armadillo coactivates transcription driven by the product of the Drosophila segment polarity gene dTCF. Cell 1997;88:789–99.
24. Cheng L, Itoh K, Lemmon V. L1-mediated branching is regulated by two ezrin-radixin-moesin (ERM)-binding sites, the RSLE region and a novel juxtamembrane ERM-binding region. J Neurosci 2005;25:395–403.
25. Nishimura K, Yoshihara F, Tojima T, et al. L1-dependent neuritogenesis involves ankyrin B that mediates L1-CAM coupling with retrograde actin flow. J Cell Biol 2003;163:1077–88.
26. Gavard J, Lambert M, Grosheva I, et al. Lamellipodium extension and cadherin adhesion: two cell responses to cadherin activation relying on distinct signalling pathways. J Cell Sci 2004;117:257–70.
27. Garcia-Vallejo JJ, Van Het HB, Robben J, et al. Approach for defining endogenous reference genes in gene expression experiments. Anal Biochem 2004;329:293–9.
28. Sadot E, Simcha I, Shtutman M, Ben Ze'ev A, Geiger B. Inhibition of β-catenin-mediated transactivation by cadherin derivatives. Proc Natl Acad Sci U S A 1998;95:15339–44.
29. Khodjakov A, Rieder CL. Imaging the division process in living tissue culture cells. Methods 2006;38:2–16.
30. Omelchenko T, Vasiliev JM, Gelfand IM, Feder HH, Bonder EM. Rho-dependent formation of epithelial "leader" cells during wound healing. Proc Natl Acad Sci U S A 2003;100:10788–93.
31. De Angelis E, Brummendorf T, Cheng L, Lemmon V, Kenwrick S. Alternative use of a mini exon of the L1 gene affects L1 binding to neural ligands. J Biol Chem 2001;276:32738–42.
32. Dickson TC, Mintz CD, Benson DL, Salton SR. Functional binding interaction identified between the axonal CAM L1 and members of the ERM family. J Cell Biol 2002;157:1105–12.
33. Panicker AK, Buhusi M, Erickson A, Maness PF. Endocytosis of β1 integrins is an early event in migration promoted by the cell adhesion molecule L1. Exp Cell Res 2006;312:299–307.
34. Pishvaian MJ, Feltes CM, Thompson P, et al. Cadherin-11 is expressed in invasive breast cancer cell lines. Cancer Res 1999;59:947–52.
35. Nieman MT, Prudoff RS, Johnson KR, Wheelock MJ. N-cadherin promotes motility in human breast cancer cells regardless of their E-cadherin expression. J Cell Biol 1999;147:631–44.
36. Katafiasz BJ, Nieman MT, Wheelock MJ, Johnson KR. Characterization of cadherin-24, a novel alternatively spliced type II cadherin. J Biol Chem 2003;278:27513–9.
37. Locascio A, Nieto MA. Cell movements during vertebrate development: integrated tissue behaviour versus individual cell migration. Curr Opin Genet Dev 2001;11:464–9.
38. Davis MA, Ireton RC, Reynolds AB. A core function for p120-catenin in cadherin turnover. J Cell Biol 2003;163:525–34.
39. Conacci-Sorrell M, Simcha I, Ben Yedidia T, et al. Autoregulation of E-cadherin expression by cadherin-cadherin interactions: the roles of β-catenin signaling, Slug, and MAPK. J Cell Biol 2003;163:847–57.
40. de Rooij J, Kerstens A, Danuser G, Schwartz MA, Waterman-Storer CM. Integrin-dependent actomyosin contraction regulates epithelial cell scattering. J Cell Biol 2005;171:153–64.
41. Maretzky T, Schulte M, Ludwig A, et al. L1 is sequentially processed by two differently activated metalloproteases and presenilin/gamma-secretase and

regulates neural cell adhesion, cell migration, and neurite outgrowth. Mol Cell Biol 2005;25:9040–53.
42. Voura EB, Ramjeesingh RA, Montgomery AM, Siu CH. Involvement of integrin α(v)β(3) and cell adhesion molecule L1 in transendothelial migration of melanoma cells. Mol Biol Cell 2001;12:2699–710.
43. Heiz M, Grunberg J, Schubiger PA, Novak-Hofer I. Hepatocyte growth factor-induced ectodomain shedding of cell adhesion molecule L1: role of the L1 cytoplasmic domain. J Biol Chem 2004;279:31149–56.
44. Islam R, Kristiansen LV, Romani S, Garcia-Alonso L, Hortsch M. Activation of EGF receptor kinase by L1-mediated homophilic cell interactions. Mol Biol Cell 2004;15:2003–12.
45. Lilien J, Balsamo J. The regulation of cadherin-mediated adhesion by tyrosine phosphorylation/dephosphorylation of β-catenin. Curr Opin Cell Biol 2005;17:459–65.
46. Capp JP. Stochastic gene expression, disruption of tissue averaging effects and cancer as a disease of development. Bioessays 2005;27:1277–85.
47. Thiery JP, Chopin D. Epithelial cell plasticity in development and tumor progression. Cancer Metastasis Rev 1999;18:31–42.
48. Kamiguchi H, Lemmon V. Neural cell adhesion molecule L1: signaling pathways and growth cone motility. J Neurosci Res 1997;49:1–8.
49. Chausovsky A, Bershadsky AD, Borisy GG. Cadherin-mediated regulation of microtubule dynamics. Nat Cell Biol 2000;2:797–804.
50. Kajiyama H, Kikkawa F, Khin E, et al. Dipeptidyl peptidase IV overexpression induces up-regulation of E-cadherin and tissue inhibitors of matrix metalloproteinases, resulting in decreased invasive potential in ovarian carcinoma cells. Cancer Res 2003;63:2278–83.
51. Arlt MJ, Novak-Hofer I, Gast D, et al. Efficient inhibition of intra-peritoneal tumor growth and dissemination of human ovarian carcinoma cells in nude mice by anti-L1-cell adhesion molecule monoclonal antibody treatment. Cancer Res 2006;66:936–43.

# Cell Adhesion Molecule L1 Disrupts E-Cadherin-Containing Adherens Junctions and Increases Scattering and Motility of MCF7 Breast Carcinoma Cells

Michael Shtutman, Elina Levina, Patrice Ohouo, et al.

*Cancer Res* 2006;66:11370-11380.