Cell–Cell Contacts Mediated by E-Cadherin (Uvomorulin) Restrict Invasive Behavior of L-Cells

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Abstract. L-cells were cotransfected with plasmids coding for mouse E-cadherin (uvomorulin) and the neoprophototransferase gene, and stable transfectants expressing E-cadherin at the cell surface were selected and cloned. Control transfection was done with the neoprophototransferase gene alone. The invasive migration of transfected and untransfected L-cells into three-dimensional collagen gels was then analyzed. L-cells not expressing E-cadherin migrated efficiently into the gels, whereas invasion of the E-cadherin-expressing L-cells was restricted in a cell density dependent manner. At sparse density, when the cells exhibited little cell–cell contacts, no difference was observed between the level of invasion of the cadherin-expressing cells and the control cells. However, with increasing cell density, decreasing amounts of the cadherin-expressing cells but increasing amounts of the control cells migrated into the gels. At confluent density hardly any cadherin-expressing cells were able to migrate into the gels. The inhibition of the invasion of the cadherin-expressing cells could be reverted if confluent cells were cultured in the presence of monoclonal antibodies against E-cadherin. Since the expression of E-cadherin did not influence the invasive mobility of single cells, these results indicate that E-cadherin-mediated cell–cell contacts inhibited invasive cellular migration. Time-lapse videocopy and studies of cell migration from a monolayer into a cell-free area demonstrated that the restricted invasion could be explained by contact inhibition of cell movement of the cadherin-expressing cells.

The formation and maintenance of structure and function of tissues depend on specific and regulated three-dimensional organizations of the component cells and extracellular matrix constituents. Although the understanding of the mechanisms governing tissue organization remains as being one of the major unsolved mysteries in metazoan biology we know that processes such as homotypic and heterotypic cell–cell adhesion, cellular interactions with extracellular matrix components, and cellular motility are central in tissue formation, tissue segregation, and maintenance of tissue boundaries. Cell–cell adhesion, that has a key role in this context, is mediated by cell adhesion molecules (CAMs), and it has been demonstrated that several CAMs are important for tissue formation during the embryonic development of chickens and mice (8, 24, 32). The most well-characterized CAMs in this respect are N-CAM and the cadherins (E-cadherin, N-cadherin, P-cadherin, and L-CAM). N-CAM is a calcium-independent molecule belonging to the immunoglobulin superfamily (8). The cadherins are calcium-dependent CAMs that mediate strong cell–cell binding in which interactions with actin-containing microfilaments are important (32). It has recently been demonstrated that differential expression of cadherins lead to sorting out of mixed cell populations (9, 22), which is crucial for tissue segregation.

Perturbation of proper intercellular organization in the tissues causes malfunction. This is most dramatic in tumor invasion and metastasis, which not only involve abnormal organization within a tissue, but also a breakdown of tissue boundaries. Cellular invasion can be defined as the intrusion of one tissue into the space occupied by a second tissue. Thus, invasion and tissue segregation are opposite modes of behavior. A better understanding of the mechanisms governing tissue formation and segregation is accordingly not only of fundamental biological importance, but would also contribute to the solution of a major medical problem, since invasive growth and metastasis are the chief causes of death from cancer.

Tumor cell invasion and metastasis are extremely complex phenomena in which among other things various cellular adhesive interactions, cellular motility, responsiveness to local growth factors, and enzymatic degradation and remodeling of the extracellular matrix play important roles (15, 18). It is believed that one of the primary events in invasion of solid tumors is release of cells from the primary tumor. Such a release may involve changes in intercellular adhesion that restricts cellular migration and is responsible for holding cells together in normal tissues.

Over the years a number of studies of the adhesive properties of malignant and transformed cells have been published, but no conclusive picture of the relationship of adhesion to tumor growth and metastasis has emerged from these studies (6, 35). Recently, however, Behrens et al. published a study where they demonstrated that perturbation of E-cadherin (uvomorulin) by antibodies led to invasion of a small propor-
Collagen (type I) was extracted from rat tail tendons and purified according to standard procedures (28). The purified collagen was dialyzed against 0.05% (vol/vol) acetic acid and stored at 4°C. Protein concentration was determined by the procedure of Lowry et al. (17) with bovine serum albumin as a standard.

Cell culture dishes (35 mm diam) were coated with collagen type I as previously described (23). Briefly, 100 μg of collagen from the stock solution was added to each dish containing 2 ml of medium A (137 mM NaCl, 4.7 mM KCl, 0.6 mM MgSO₄, 1.2 mM CaCl₂, 10 mM Hepes, pH 7.4). The dishes were incubated for 30 min at 37°C, rinsed extensively with medium A, and used for experiments.

Collagen gels were formed by mixing 8.5 ml of the collagen stock solution (0.5 mg/ml) with 1 ml of 10X MEM and 0.5 ml of 4.4% (wt/vol) NaHCO₃. 2 ml of this solution was rapidly pipetted into 35-mm tissue culture dishes and incubated in 5% CO₂/95% air at 37°C. Gels formed in the dishes within 5 min. The gel-containing dishes were incubated at 37°C for 24 h before being used in cell invasion assays.

**Invagination Assay**

Cells were brought into suspension by trypsin treatment and counted in a hemocytometer. They were taken up in MEM-FCS, adjusted to the desired concentration, and transferred to the collagen gel–containing dishes in a final volume of 2 ml/dish. The dishes were incubated at 37°C in 5% CO₂/95% air for various times. They were inspected every day and the number of cells inside the collagen gels was determined.

The number of cells within the gels was determined in two ways according to Schor et al. (31) with slight modifications. Method I: The cultures were examined with phase-contrast optics using a Nikon Labophot inverted microscope equipped with a graticule in the eyepiece defining an area of 0.24 × 0.24 mm². For counting cells within the complete thickness of the gel within this projected area 25–50 regions in each of duplicate dishes were selected at random by moving along five parallel lines across the whole dish. The mean, SD, and significance levels for pairwise determinations (according to t test) were calculated by the Stat View program for Macintosh computers. Method II: The cells growing on the surface of the gel were removed by a short trypsin treatment (2 ml of 0.5 mg/ml trypsin, 2 mM EDTA in PBS for 10 min at 37°C), the dishes were washed with 3 ml of Hank’s balanced salt solution, and the collagen gels were digested by collagenase (2 ml of 0.2 mg/ml collagenase [type I, Worthington Biomedical Corp., Freehold, NJ] in serum-free MEM for 30 min). The cells released by the collagenase digestion were diluted with PBS and counted in a Coulter counter (model ZBI; Coulter Electronics, Hialeah, FL).

**Antibodies**

A hybridoma cell line, DECA-M-1 (34), producing monoclonal antibodies against E-cadherin was kindly given to us by Dr. Rolf Kemler, Max Planck Institute, Freiburg, Germany. The cells were cultured in MEM, 5% FCS, penicillin (100 U/ml), and streptomycin (100 μg/ml). Antibodies were isolated from the medium by affinity chromatography on protein G-Sepharose Fast Flow (Pharmacia, Uppsala, Sweden) according to the manufacturer. A monoclonal antibody, F7 (2), against the cell adhesion molecule C-CAM was isolated from culture medium (RPMI 1640, 5% FCS) by affinity chromatography on protein A-Sepharose (Pharmacia) according to the manufacturer. C-CAM is a cell–cell adhesion molecule belonging to the immunoglobulin superfamily (3) and occurs in liver, a variety of epithelia, vessel endothelia, and platelets (26). The antibodies were dialyzed against PBS and were concentrated by ultracentrifugation with a PM-10 Diaflo Filter (Amicon Corp., Danvers, MA). Antibody concentration was determined by UV absorption at 280 nm assuming an absorption coefficient of 1.4 per mg of protein at 1-cm path length.

**Immunoblotting**

Cells in tissue culture flasks were washed with PBS and harvested by scraping with a rubber policeman. The cells were collected by centrifugation, solubilized in boiling SDS-sample solution (100 μl/10⁶ cells), reduced and subjected to SDS-PAGE (an equivalent of 5 × 10⁶ cells per lane) as previously described (25). The separated proteins were electrophoretically transferred to nitrocellulose sheets (25), which were blocked in 5% defatted dry milk dissolved in TBS and 0.05% Tween 20. The filters were then incubated sequentially with monoclonal antibodies and secondary antibodies conjugated with alkaline phosphatase and were developed with nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) (5).

**Immunofluorescence Microscopy**

Cells were grown on coverslips, fixed with 2% paraformaldehyde, permeabilized in 0.1% Triton X-100, and incubated with the monoclonal antibody...
DECMA-1. After washing with 1% BSA in medium A, FITC-conjugated rabbit anti-rat immunoglobulin (DAKOPATT S. Copenhagen, Denmark) was added. The specimens were washed, mounted in 90% glycerol/10% PBS, and analyzed in a Nikon Labophot microscope equipped with epi-illumination. Photographs were taken using Kodak TRI-X film.

Results

Quantitation of Cell Invasion into Collagen Gels

Cells on and within the transparent collagen gels were observed in an inverted microscope equipped with bright-field and phase-contrast illumination (Fig. 1). Normal L-cells seeded on the collagen gel surface migrated deep into the gel (Fig. 1), and this invasion could be quantified by two independent methods. Method I was based on counting the number of cells within a specified volume of the collagen gel, that was observed in the inverted microscope. The height of the gel volume corresponded to the entire depth of the gel, and the horizontal borders of the volume was defined by a graticule in the microscope eyepiece. In Method II the cells growing on the gel were first removed by trypsin treatment. The cells within the collagen gel were then recovered by collagenase digestion and counted in a Coulter counter. There was an excellent agreement in the number of invading cells given by the two methods, and Method I was then used in all further analyses of cellular invasion into collagen gels. The data are given as mean and 1 SD of the number of invading cells per cm².

The influence of collagen concentration in the gels on invasion of normal L-cells was analyzed. Maximal invasion occurred at collagen concentrations between 1 and 2 mg/ml. All comparative experiments were done at a collagen concentration of 1.3 mg/ml.

When seeded and incubated under standard conditions normal L-cells could be seen inside the gels after 2 d. The number of cells within the gels increased rapidly for ~10 d after which a plateau level was approached (Fig. 4). At this stage the number of cells within the gels amounted to ~20% of the cells in the confluent layer on the top surface of the gels.

Figure 1. L-cells on and in collagen gels. Normal, untransfected L-cells (L0) seeded at confluent density (2 × 10⁶ cells/35-mm dish) on a collagen gel were cultured for 6 d and photographed by phase-contrast microscopy. (a) Cells growing on the surface of the gel. (b) Cells that had invaded just below the surface layer. (c) Cells that had invaded to approximately one-half of the height of the collagen gel. Bars, 50 μm.

Transfection and Characterization of Transfected L-cells

L-cells were cotransfected with pBATEM2 and the neophosphotransferase gene, or with the neophosphotransferase gene alone for control experiments. Stable transfectants were selected by growth in the presence of genetecin (G 418). These cells were then analyzed by immunofluorescence, using the monoclonal antibody DECMA-1 which recognizes E-cadherin. Whereas none of the cells transfected with the neophosphotransferase gene alone were positive the majority of the pBATEM2-transfected cells exhibited cell surface staining to varying degrees. The E-cadherin-expressing cells were cloned and several independent E-cadherin positive clones were isolated. Two such clones, 12H and 13, which exhibited a strong staining intensity for E-cadherin (Fig. 2), were selected for invasion analysis.

The untransfected and the transfected L-cells were characterized with respect to proliferation kinetics, growth pattern, and morphology when cultured both on plain tissue culture dishes and on collagen-coated dishes. There were no differences in proliferation kinetics between the various types of L-cells. Both the untransfected L-cells (L0-cells), the neo-transfected cells (Neo-cells), and the cadherin-expressing cells (12H and 13) grew with a doubling time of ~24 h on plain dishes (data not shown). On collagen-coated dishes the proliferation rate was a little faster, but there was still no difference between the different types of L-cells. Furthermore, the different cell types reached the same cell density at confluency, and all of them remained as monolayers without any piling up of cells. However, for all cell types the density at confluency was a little lower on collagen-coated dishes (2 × 10⁶ cells/cm²) than on plain dishes (4 × 10⁵ cells/cm²). This is in agreement with the observation that the cells showed a slightly larger degree of spreading on the collagen-coated dishes meaning that each cell occupied a larger area on this substratum.

The growth pattern of the 12H- and 13-cells differed from the Neo-cells or the L0-cells. Whereas L0- and Neo-cells grew as single cells without obvious cell-to-cell contact for-
mation 12H- and 13-cells formed small colonies of cells exhibiting extensive cell–cell contacts. At sparse densities the morphology of single cells was similar for both the control cells and the cadherin-expressing cells (Fig. 2). At confluence the LO- and the Neo-cells still appeared largely as individuals without extensive cell cell contact formation (Fig. 2). The cadherin-expressing cells on the other hand formed a sheet of polygonal, epithelial-like coherent cells (Fig. 2). These differences between the cadherin-expressing cells and the other L-cells appeared both on plain dishes and on collagen-coated dishes. Immunofluorescence staining showed that E-cadherin was localized to the cell–cell contact areas in the coherent cells (Fig. 2). When the cells were grown on collagen gels instead of collagen-coated dishes or plain dishes the differences in morphology were less pronounced. At confluency the cells were more rounded, but all the cell types remained as monolayers at the same density (2 × 10^6 cells/cm²). No piling up of any cell type was observed on the collagen gels.

Expression of E-cadherin in the transfected cells was verified by immunoblotting. As demonstrated in Fig. 3 the characteristic 120-kD protein was found both in the 12H- and the 13-cells, whereas the LO-cells and the Neo-cells lacked this molecule.

**Invasive Behavior of Transfected Cells**

The invasive behavior of the E-cadherin-expressing cells was compared with that of the control cells. Clear differences in the amount of invading cells were observed when the cells were seeded at confluent density (Fig. 4). While both the LO- and the Neo-cells invaded the gels to a high degree during a period of 8 d few of the 12H- or 13-cells migrated into the gels. There was no statistically significant difference between the invasive potential of the LO-cells and the Neo-cells. Likewise no statistically significant difference was seen between the 12H- and 13-cells. However, the differences between the LO-cells and the Neo-cells on the one hand and the 12H- and the 13-cells on the other hand were highly significant (P < 0.0001).

The influence of the seeding density on the degree of invasion was then analyzed. The rationale behind this experiment was that if the restricted invasion of the cadherin-expressing cells was due to cell–cell contact formation, there would be a dependence on cell density (see Discussion). Indeed a dramatic effect on the degree of invasion was observed. In Figs. 5 and 6 the number of invading cells are shown at two different times, 8 and 13 d after seeding, respectively. The cells were seeded at three different densities: 2 × 10^6 cells/gel (confluent density), 1 × 10^6 cells/gel, and 0.2 × 10^6 cells/gel, respectively. At the lowest seeding density (0.2 × 10^6 cells/gel) the same number of cells of both the cadherin-expressing and cadherin-nonexpressing cells moved into the gels. The number of LO- and Neo-cells that migrated into the gels increased with increasing seeding density. However, the 12H- and 13-cells behaved in a completely opposite manner; the number of these cells that invaded the gels decreased with increasing seeding density.

**Effects of Antibodies against E-cadherin on Cellular Invasion**

If the restricted invasive behavior of the cadherin-expressing cells was due to formation of cadherin bonds, blocking of
such bond formation should revert the cells to become more invasive. To test this we performed the invasion experiments in the presence of anti-E-cadherin monoclonal antibodies (DECMA-1), that are known to block E-cadherin-mediated cell adhesion (34). As demonstrated in Fig. 7 this antibody did cause 12H-cells to invade the collagen gels to the same extent as Neo-cells. Another monoclonal antibody (F7) against the cell adhesion molecule C-CAM, which is not expressed in L-cells, had no effect on the invasion of the E-cadherin-expressing cells or the control cells (Fig. 7).

**Motility and Locomotion of Transfected Cells**

The collagen gel invasion experiments demonstrated that cell contacts mediated by E-cadherin inhibited cellular invasion. To investigate if this effect might be due to contact inhibition of cell movement we analyzed the motility of the various cell types. First we used time-lapse videomicroscopy to compare the motility of LO-cells, Neo-cells, and 12H-cells, both on plain dishes and on collagen-coated dishes. In sparse cultures the motility patterns of these cells were similar. They moved on the surface as individual cells and had active membrane motility. The membrane motility of the LO- and the Neo-cells was not affected when the cells collided with other cells. However, when the 12H-cells came into contact with other cells the membrane movement in the contact area was paralyzed. When the cultures became confluent a significant difference in the motility pattern of the cadherin-expressing cells and the control cells was observed. The LO- and the Neo-cells continued to move and to change places with each other in the confluent monolayer. The 12H-cells on the other hand became completely immobilized, and did not exchange places with their neighbors. These results thus demonstrate that the cadherin-expressing 12H-cells, in contrast to the LO-cells and the Neo-cells, exhibited a true contact inhibition of locomotion. The differences in the motility and migration pattern between the control cells, and the 12H-cells were observed both on plain dishes and on collagen-coated dishes.

We also used a wound assay to analyze the locomotory behavior of the various types of L-cells. In this assay confluent cell monolayers were scratched to form a uniform cell-free
area of defined width. The cultures were then inspected regularly to determine how long it took before the wounds were covered with cells. As demonstrated in Fig. 8 the Neo-cells covered the wound in a much shorter time than the 12H-cells. On plain dishes the Neo-cells had covered the wound completely 18 h after scratching (Fig. 8 a) whereas a broad zone still was without cells in the 12H-cell culture (Fig. 8 b). On collagen-coated dishes it took the Neo-cells 10 h to cover the wound (Fig. 8 c), at which time-point a cell-free area still remained in the 12H-cell cultures (Fig. 8 d). Since the Neo-cells and the 12H-cells had identical proliferation rates this difference thus reflects a lower locomotory activity of the latter cell type.

Discussion

In the present investigation we have used transfection technology to analyze the relationship between expression of the cell adhesion molecule E-cadherin (also known as uvomorulin) and cellular invasive behavior. Previous work from the laboratories of Takeichi and Edelman has demonstrated that transfection with plasmids coding for cell adhesion molecules renders nonadhesive cells to become adhesive (19, 20). Takeichi's group showed that L-cells expressing E-cadherin aggregated with each other and grew as epithelial-like colonies on plain tissue culture dishes. The L-cells that we transfected, and which showed a stable expression of E-cadherin, behaved in the same way. Confluent cell layers of closely associated polygonal cells were formed, and E-cadherin was highly concentrated in the cell–cell borders.

Normal L-cells, which do not express E-cadherin and which exhibit very little cell–cell adhesion (20), migrated effectively into collagen gels both when seeded at sparse and confluent densities. In contrast the cadherin-expressing L-cells showed a drastically reduced migration into such gels when seeded at confluent density. This change in behavior was clearly a function of the expression of E-cadherin since transfection under identical conditions with the neophosphotransferase gene alone had no effect on the infiltrative migration. Furthermore, it seemed to be due to the exposure of E-cadherin on the extracellular face of the cell surface, since antibodies against E-cadherin were able to revert the cells to become infiltrative.

The E-cadherin-expressing L-cells had the same proliferation rate and saturation density at confluency as the untransfected cells or the cells transfected with only the neophosphotransferase gene. All these cell types formed simple monolayers, with no tendencies to pile up in multilayered configurations, both on plain dishes, collagen-coated dishes and on collagen gels. Thus, the observed differences in the invasive behavior between the different cell types were not due to variations in the proliferation rates or pattern formation. Other factors that might be responsible for the altered invasive behavior of the E-cadherin-expressing cells are (a) modification of the collagen gel matrix, (b) modification of the inherent cellular motility, and (c) modification of cell adhesion.

Modification of the gel matrix might occur if the cells secrete collagenase. However, there were no signs of either macroscopic degradation or dissolution of the collagen gel matrix over a period of >15 d with either control cells or E-cadherin-expressing L-cells. Furthermore, the cells were cultured in the presence of fetal calf serum, that contains collagenase inhibitors (11). It has also been demonstrated previ-
ously that invasion of several other cell lines into collagen gels proceed without collagen degradation (29, 30). Thus, it seems unlikely that matrix degradation was responsible for the observed invasion.

Cellular motility is influenced in various ways, e.g., by autocrine motility factors (10, 16) and by adhesive interactions with the extracellular matrix on which the cells migrate (33). Although these parameters were not analyzed the time-lapse videomicroscopy experiments demonstrated that there was no difference in the inherent single cell motility between the cadherin-expressing cells or the control cells. This conclusion was further strengthened by the observation that these cells invaded the collagen gels to the same extent when they were seeded at sparse density.

Analysis of the invasive behavior at different cell densities indicated that altered cell–cell adhesion was the main factor responsible for the inhibition of invasion of the cadherin-expressing cells. Thus, at sparse density, when the cells appeared as separate units, there was no difference in the infiltrative migration of cadherin-expressing and cadherin-nonexpressing cells. With increasing cell density the number of nonexpressing cells that migrated into the collagen gels increased, which reflects the larger number of cells added to the gels. However, with increasing cell density there was a decrease in the number of cadherin-expressing cells that migrated into the gels. At confluence very few cadherin-expressing cells entered the gel. Since increasing cell density increases the extent of cell–cell contacts the most plausible explanation to the density-dependent abrogation of infiltrative migration is E-cadherin-mediated cell–cell adhesion. This conclusion was further strengthened by the ability of the antibodies against E-cadherin to restore the invasive potential of confluent cadherin-expressing cells. Accordingly, it was not the expression of E-cadherin on the cell surface per se that changed the infiltrative migration but rather the contact formation between the cells that was mediated by E-cadherin.

These results are in agreement with and extend those of Behrens et al. (4). They found that antibodies against E-cadherin made normal MDCK cells to invade collagen gels and heart explants. However, only 1–2% of the cells became invasive and no attempts were made to correlate the invasive behavior to cell contact formation.

The L-cells that infiltrated into the collagen matrix appeared largely as single cell units of elongated, spindle-shaped morphology, suggesting that the infiltration is a function of active cellular locomotion whereby the cells migrate on the collagen fibers making up the gel. Thus, the E-cadherin-mediated inhibition of invasion might be due to contact inhibition of cellular locomotion. To provide further evidence for this hypothesis the loco motory properties were studied by time-lapse videomicroscopy and by a wound assay. Both assays showed unambiguously that the locomotion of the cadherin-expressing L-cells, but not of the control cells, was significantly inhibited by contact formation. It remains to be seen if this contact inhibition is due only to increased cell–cell adhesion making it more difficult for the cells to break loose from each other, or if it is a result of a specific paralysis of the motility machinery. The known interaction of E-cadherin with cytoplasmic microfilaments (21) and the recently identified molecules (27) that might mediate cadherin–microfilament binding indicate that cadherin-mediated contact inhibition is a result of a specific influence on the motility machinery.

A characteristic feature of contact–inhibition of locomotion of fibroblasts is that complete inhibition occurs only when a cell is surrounded on all sides by other cells (13). When a migrating fibroblast in a nonconfluent layer collides with another cell the locomotion is stopped and the ruffling activity in the contact area ceases. However, after some time a new motility center develops in a free area of the cell, which then starts migrating in another direction and detaches from the cell with which it collided (13). Thus, one should expect maximal inhibition of cell locomotion in a confluent layer and only partial inhibition in subconfluent layers.

Since active locomotion requires stimuli of various kinds it seems likely that cell locomotion is regulated by a balance of motility stimulatory and motility inhibitory factors. It has been demonstrated that small variations in the concentration of cell adhesion molecules result in large differences of the rate and strength of adhesion (14). Accordingly, an increasing amount of E-cadherin on the cell surface should cause a stronger and more pronounced inhibition of cell locomotion. This offers an explanation as to why we did not observe a total inhibition of invasion of the E-cadherin-expressing cells at confluence. The motility inhibitory action mediated by E-cadherin was not large enough to completely counterbalance the motility of all the cells. Interestingly we observed that the fraction of the cells that still invaded the collagen matrix expressed less cadherin and showed less extensive cell contact formation than the cells that remained on the surface of the matrix (data not shown).

The present results have important implications for our understanding of the processes leading to formation of normal tissues. It has already been demonstrated that adhesive interactions mediated by cadherins can result in sorting out (9, 22). It is also known that cell migration is an important factor (33). Now we demonstrate that cadherin activity at the cell surface not only can affect the composition of the cellular aggregates that are formed, but also can control the cellular motility. Thus, cadherins seem to have a dual function in regulating tissue segregation.

In view of our results and those of Behrens et al. (4) it seems reasonable to conclude that cell contact interactions mediated by E-cadherin counteract cellular invasion. Thus, a loss of this adhesive function may be an important step in the acquisition of an invasive phenotype in epithelial cells. It will be important to investigate if the prevention of invasiveness is specific for E-cadherin or if other cell adhesion molecules as well have similar regulatory properties. This can be done with the present model system, which also can be expanded to study other parameters that are involved in tumor invasion.

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