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Influence of tumor-associated E-cadherin mutations on tumorigenicity and metastasis

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In this study, we investigated whether tumor-associated E-cadherin mutations impair the tumor-suppressive function of the cell adhesion molecule and influence metastasis formation in a severe combined immunodeficiency mouse model. The investigated E-cadherin mutations were in frame deletions of exons 8 (*del 8*) or 9 (*del 9*) and a point mutation in exon 8 (*p8*). Transfected human MDA-MB-435S carcinoma cells stably expressing wild-type (*wt*) or mutant E-cadherin were injected into the mouse mammary fat pad. Mice transplanted with *wt* E-cadherin transfectants developed significantly smaller tumors than animals transplanted with the E-cadherin-negative parental cell line. Animals transplanted with *del 9* or *p8* E-cadherin transfectants produced medium size tumors, indicating that these mutations impair the tumor-suppressive function of E-cadherin. In contrast, mice transplanted with *del 8* E-cadherin transfectants developed tumors of approximately the same sizes as animals transplanted with *wt* E-cadherin expressing cells. Lung metastases were induced by all cell lines without significant differences. Immunohistochemical analysis of E-cadherin expression in the tumors revealed a heterogeneous staining pattern, indicating loss or down-regulation of E-cadherin in some tumor cells. Metastases were completely negative for E-cadherin. Our data suggest that the type of mutation determines whether the tumor-suppressive function of E-cadherin is impaired.

Introduction

The adhesive function of E-cadherin depends on the association with catenins, which link the adhesion molecule to the actin cytoskeleton (1,2). Epidemiological studies have revealed a relationship between loss of E-cadherin and/or catenin expression and invasive tumor growth in a variety of tumors (3–6), including gastric (7) and breast carcinomas (8,9). *In vitro* studies with human cancer cell lines revealed that expression of E-cadherin results in a more differentiated phenotype and loss of invasiveness (10–12). E-cadherin has therefore been proposed to act as a tumor and invasion suppressor.

E-cadherin expression is under transcriptional control of the negatively regulating transcription factors snail and sip1 (13–16) and can be silenced by DNA hypermethylation (17–18). Beside these reversible mechanisms for down-regulation of E-cadherin expression, somatic mutations of the E-cadherin gene have been described for breast, endometrium, gastric, ovary and thyroid carcinomas (4). Inactivating germline E-cadherin mutations have been identified in families with diffuse-type gastric carcinoma (19–22). These genetic alterations comprise missense mutations, splice site mutations and truncation mutations. We and other groups have identified splice site E-cadherin mutations in 50% of diffuse-type gastric carcinomas (23–28). The most frequent mutations were in frame deletions of exons 8 or 9, which affect putative calcium binding sites within the extracellular domain of E-cadherin (29). Expression of these mutant E-cadherin variants in MDA-MB-435S carcinoma cells resulted in decreased cellular adhesion and increased cellular motility as compared with the *wt* E-cadherin molecule (29–31) and interfered with the proliferation-suppressive function of E-cadherin (32).

Tumor-derived E-cadherin mutations have been shown to lead to a loss of function on one side (loss of adhesive properties and proliferation-suppressive function) and a gain of function on the other side (increase in cellular motility), both of which are potentially relevant for tumor and metastasis formation. To determine whether E-cadherin mutations play a role in tumor development, we investigated tumor and metastasis formation of parental, *wt* and mutant E-cadherin expressing MDA-MB-435S carcinoma cells in a severe combined immunodeficiency (SCID) mouse model.

Materials and methods

Cell cultivation

The human E-cadherin-negative cell line MDA-MB-435S (ATCC, Manassas, VA) and the wild-type and mutant E-cadherin-cDNA transfected derivatives that were established by Handschuh *et al*. (29) were grown in Dulbecco's modified Eagle medium (Life Technologies, Eggenstein, Germany) supplemented with 10% fetal calf serum (PAN Biotech, Aidenbach, Germany) and penicillin–streptomycin (50 IU/ml and 50 μg/ml; Life Technologies) at 37°C and 5% CO_2.

Flow cytometry

Cells were harvested with versene and 5×10^5 cells were incubated with 4 μg/ml monoclonal antibody directed to E-cadherin SHE78-7 (Alexis Deutschland, Grünberg, Germany) for 1 h on ice in phosphate-buffered saline, washed with 0.1% sodium azide and 0.1% bovine serum albumin (Sigma, Deisenhofen, Germany) and stained with DTAF-conjugated anti mouse IgG (Jackson

Abbreviations: *del 8* E-cadherin, E-cadherin with deletion of exon 8; *del 9* E-cadherin, E-cadherin with deletion of exon 9; *p8* E-cadherin, E-cadherin with point mutation in exon 8; SCID, severe combined immunodeficiency; *wt* E-cadherin, wild-type E-cadherin.

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ImmunoResearch Laboratories, West Grove, PA) for 1 h on ice. Purified mouse IgG1 (Pharmingen, Heidelberg, Germany) was used as κ immunoglobulin isotype control. Cells were analyzed on a Beckman Coulter Epics XL (Beckman Coulter, Krefeld, Germany).

Injection of MDA-MB-435S transfectants into the mammary fat pads of SCID mice

SCID bg mice (Harlan Winkelmann, Borchen, Germany) were housed under pathogen-free conditions. Mice were anesthetized with fentanyl/dormitor/dormicum (0.05/0.5/1 mg/ml; Janssen-Cilag, Neuss, Germany; Pfizer, Karlsruhe, Germany; Hoffmann-La Roche, Grenzach-Wyhlen, Germany). Then a 5 mm incision was made in the skin to expose the mammary fat pad and 5×10^6 cells were injected into the fad pad. The wound was closed with vicryl 6/0 (Johnson & Johnson, Brussels, Belgium) and the animals were subcutaneously injected with 0.5 ml 5% glucose solution (B.Braun, Melsungen, Germany). Tumor growth (tumor length × width in mm^2) was measured twice per week. Mice were killed when the primary tumor had reached an approximate size of 10 mm after 38 and 46 days. Primary tumors were prepared and the tumor volumes in mm^3 calculated. Primary tumors and organs were formalin-fixed and paraffin-embedded and analyzed histologically for metastases. Animals were handled according to the German animal protection guidelines.

Histological and radiological analysis

Four to five micrometer thick sections from the primary tumors, as well as other organs including heart, lungs, brain, thymus, colon, bone marrow, lymph nodes, spleen, kidney and liver were cut and stained with hematoxylin and eosin (H&E). All sections were reviewed by two pathologists (M.K. and L.Q-M). The size of the metastases was measured with a grid ocular. All animals were radiologically analyzed in order to detect bone metastases (Cabinet X-ray system, faxitron series 43855A, Hewlett-Packard, Palo Alto, CA).

Immunohistochemical analysis

Immunohistochemistry was performed on an automated immunostainer (Ventana Medical Systems, Tucson, AZ) according to the company's protocols, with minor modifications. Formalin-fixed and paraffin-embedded *del 8* and *del 9* E-cadherin expressing MDA-MB-435S cells as well as sections from primary tumors and lungs were analyzed. After deparaffinization and rehydration, the slides were placed in a microwave pressure cooker in 0.01 mol/l citrate buffer (pH 6.0) containing 0.1% Tween 20 and heated in a microwave oven at maximum power for 30 min. The sections were cooled in Tris-buffered saline and washed in 3% goat serum for 20 min. The antibodies used included wild-type E-cadherin (Transduction Laboratories, BD Biosiences, Heidelberg, Germany, #C20820) and mutation-specific *del 8* and *del 9* E-cadherin antibodies that were produced in our laboratory and reported elsewhere (25,33). The proliferation rate was assessed with the monoclonal antibody against Ki-67 antigen (clone MiB1, Dako, Glostrup, Denmark). Appropriate positive controls were used to confirm the adequacy of the staining. A grid occular objective was used to count positive cells over 10 high-power fields (×40), and the percentage of positive cells was reported as 0–100%.

Statistical analysis

The Mann–Whitney test was performed as described by Marcus *et al*. (34). Significance was set to 5%. An exact two-sided χ^2 test was performed when appropriate.

Results

Flow cytometric investigation of the expression of wild-type and mutant E-cadherin

In the present study, we compared the tumorigenic and metastatic properties of MDA-MB-435S carcinoma cell transfectants expressing *wt* or mutant E-cadherin variants (*del 8*, *del 9* and *p8* E-cadherin) in SCID mice. *wt* and mutant E-cadherin cDNAs were cloned previously from non-tumorous gastric mucosa or from diffuse-type gastric carcinomas, respectively (29). Flow cytometric analysis of E-cadherin carried out with non-transfected, *wt* and mutant E-cadherin expressing MDA-MB-435S cells revealed that E-cadherin was expressed in all transfected cell lines in >95% of the cells (Figure 1). Expression of *wt* and mutant E-cadherin in MDA-MB-435S cell transfectants was also demonstrated by immunoblot and immunofluorescence analyses (29).

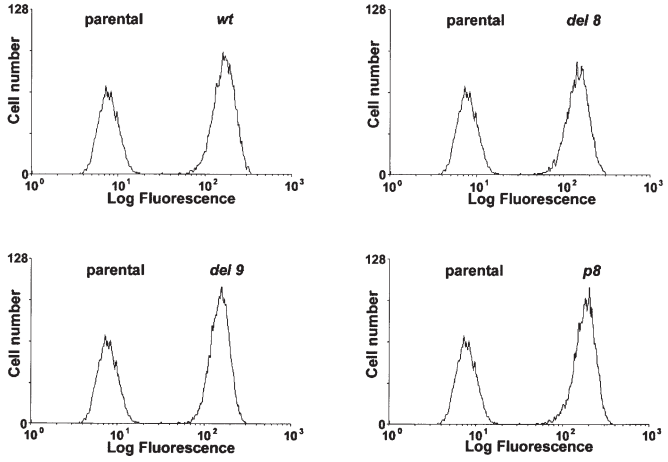

Fig. 1. Expression of *wt* and mutant E-cadherin-cDNA in MDA-MB-435S cells. Flow cytometric analysis was carried out on parental, *wt* and mutant (*del 9*, *del 8*, *p8*) E-cadherin expressing cells stained with monoclonal antibodies to E-cadherin and DTAF-conjugated anti-mouse IgG.

Injection of MDA-MB-435S transfectants into the mammary fat pads of SCID mice

Parental and transfected MDA-MB-435S cell lines were injected into the mammary fat pads of five to six SCID mice per cell line. Tumor and metastasis were observed during 38–46 days after transplantation. All mice developed tumors in the mammary fat pad. Tumor growth was measured twice per week. The growth curves of tumors derived after inoculation of parental, *del 9* and *p8* E-cadherin expressing cells were not statistically different, while tumors obtained after injection of *wt* and *del 8* E-cadherin expressing cells started to grow significantly slower than those derived from parental cells at day 35 (*wt* E-cadherin) or 28 (*del 8* E-cadherin) (Figure 2A). Two animals per cell line were killed at day 38 to investigate whether metastases were detectable (see below). The remaining mice were killed at day 46. Primary tumors were prepared and tumor volumes were calculated (Figure 2B). Tumors obtained after transplantation of *wt* E-cadherin expressing MDA-MB-435S cells were significantly smaller than those obtained after injection of non-transfected parental cells ($P = 0.008$) (Figure 2B). Different mutant E-cadherin variants had diverse effects on tumor formation: MDA-MB-435S cells expressing *del 8* E-cadherin induced tumors of similar size as cells expressing *wt* E-cadherin, whereas MDA-MB-435S cells expressing *del 9* and *p8* E-cadherin induced tumors that were smaller than those obtained after transplantation of parental cells, but larger than those obtained after injection of *wt* E-cadherin expressing cells (Figure 2B).

Metastases were identified only in lungs; their sizes ranged from a few cells to large groups of neoplastic cells. Radiological and histological analysis of all animals revealed that no bone metastases were detectable. E-cadherin-negative MDA-MB-435S cells caused lung metastases in four of five animals (80%). The incidence of lung metastasis was 80% for *wt*, 60% for *del 8* and *del 9* E-cadherin and 100% for *p8* E-cadherin. No significant differences in the number of lung metastases per animal ($P = 0.387$) nor in the areas of metastases ($P = 0.64$) were observable, neither after 38 nor after 46 days, nor when all data were combined.

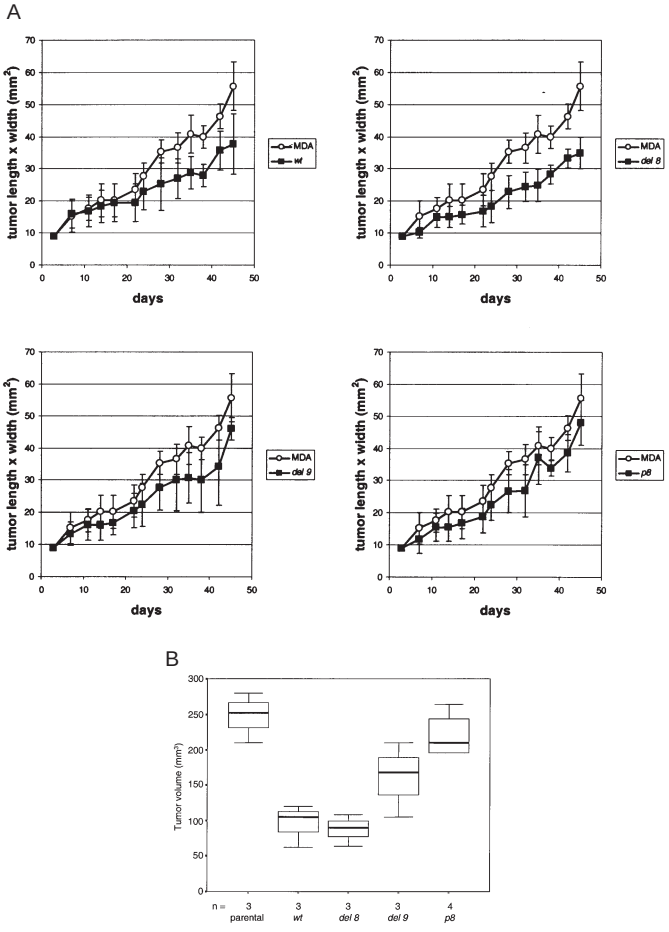

Fig. 2. Tumorigenicity and lung metastasis of parental, *wt* and mutant E-cadherin expressing MDA-MB-435S cells in SCID mice. MDA-MD-435S cells (MDA, parental, $n = 5$), *wt* ($n = 5$), *del 8* ($n = 5$), *del 9* ($n = 5$) and *p8* ($n = 6$) E-cadherin expressing cells were injected in the mammary fat pads of SCID mice. (**A**) Tumor growth was measured twice per week. At day 38, two mice per cell line were killed to determine whether metastasis formation had occurred. (**B**) At day 46, the remaining animals were killed and tumor volumes were calculated. A boxplot is shown, the thick line indicates the median, the bars show the maximum and the minimum values (Mann–Whitney test).

Histological and immunohistochemical findings

All of the tumors showed the same morphology and were composed of epithelial cells with large nuclei, prominent nucleoli and abundant cytoplasm. Karyorrhexis and focal or confluent necrosis were frequently observed, mostly in the center of the tumors (Figure 5A and B).

Expression of E-cadherin was investigated in two mice per cell line in primary tumors and metastases (Figure 3, Table I). Immunohistochemical analysis of E-cadherin in the tumors derived from parental MDA-MB-435S cells revealed complete absence of E-cadherin expression, whereas tumors derived from *wt* E-cadherin transfected cells expressed the protein in 20% of the neoplastic cells in a membranous pattern (Figure 3A and B). The expression of E-cadherin in the remaining three groups of animals inoculated with *del 8*, *del 9* and *p8* E-cadherin expressing cell lines was diverse. E-cadherin expression was noticed only in 5% of the neoplastic cells in the tumors composed of *del 8* E-cadherin cells. In contrast, tumors with *del 9* and *p8* E-cadherin mutations revealed a much higher positivity (*del 9*: 70 and 80%; *p8*: 60 and 80%) (Figure 3C–E). All lung metastases were negative for E-cadherin (Figure 3F), regardless of the cell line of origin.

In order to prove the success of the transfection, cell blocks of the original cell lines and the tumors were stained with specific antibodies against the two deletion variants (*del 8* and *del 9*) (Figure 4). The cell line with *del 8* E-cadherin revealed 100% positivity with the corresponding antibody (Figure 4A). In contrast, tumors derived from this cell line showed only rare cells with a membranous staining (Figure 4B). The cell line with *del 9* E-cadherin and the tumors derived from this cell line showed a membranous staining in the majority of the tumor cells with the specific antibody (Figure 4C and D). Lung metastases were negative for both *del 8* and *del 9* E-cadherin antibodies.

The mean proliferation rate varied from 15 to 70% in the different tumors, as measured by MiB1 staining of two tumors per cell line (Figure 5). Tumors derived from *del 8* E-cadherin expressing MDA-MB-435S cells showed the highest proliferation rate (mean 70%) (Figure 5B and E). In contrast, tumors derived from *wt* E-cadherin expressing MDA-MB-435S cells showed the lowest proliferation rate (mean 15%) (Figure 5A and D), followed by the tumors derived from parental (mean 40%) (Figure 5C), *del 9* (mean 40%) (Figure 5F) and *p8* (mean 50%) (Figure 5G) E-cadherin expressing MDA-MB-435S cells.

Discussion

The E-cadherin–catenin complex is critical for epithelial cell adhesion and maintenance of tissue integrity. Expressional abnormalities and mutational inactivation of E-cadherin are associated with a plurality of cancers and have been postulated to be implicated in tumor development and progression. Consistent with these findings, a tumor and invasion suppressor role of E-cadherin has been proposed. In this study, transplantation of parental, *wt* and mutant E-cadherin expressing MDA-MB-435S cells was performed to investigate the effect of E-cadherin mutations on tumor and metastasis formation in SCID mice. Three major observations were made: first, primary tumor sizes were dependent on the E-cadherin expression and/or mutation status. Secondly, E-cadherin expression in tumors was heterogenous, indicating down-regulation or loss of E-cadherin. Thirdly, lung metastases were completely negative for E-cadherin.

Tumor size is influenced by the E-cadherin expression and/or mutation status

Tumor volumes determined after 46 days revealed that expression of *wt* E-cadherin resulted in smaller tumor sizes in comparison with non-transfected parental cells, a finding that is consistent with the tumor-suppressive function of E-cadherin. Two of three mutant E-cadherin variants (*del 9* and *p8* E-cadherin) induced tumors that were larger than those obtained after injection of *wt* E-cadherin cadherin expressing cells. This result indicates that mutations of the E-cadherin gene cause a partial loss of the tumor-suppressive E-cadherin function. However, the effect is dependent on the type of E-cadherin mutation as *del 8* E-cadherin expressing cells induced tumors of similar size as *wt* E-cadherin expressing cells. Tumors derived from *del 8* E-cadherin expressing cells showed the highest proliferation rate of all investigated tumors, whereas tumors induced by *wt* E-cadherin expressing cells revealed only a low percentage of Ki-67 positivity. A possible explanation for the contradiction between tumor size

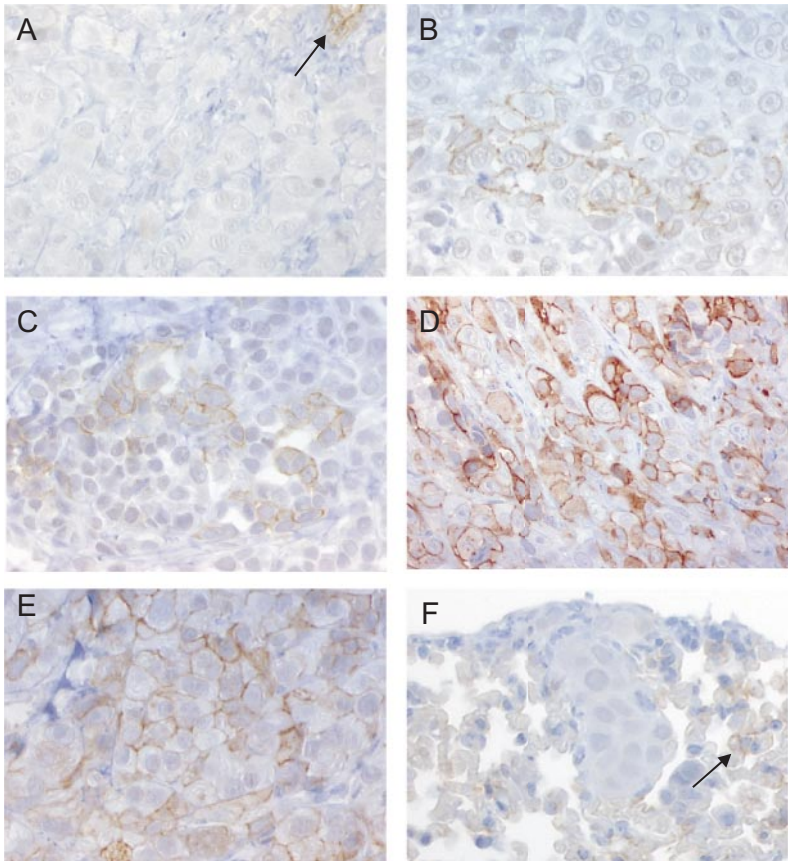

Fig. 3. Immunohistochemical study of E-cadherin in primary tumors and metastases. (**A**) Tumors derived from parental MDA-MB-435S cells were E-cadherin negative. Epithelial mice cells from residual adnexa of the skin served as internal staining control (arrow). (**B**) Tumors obtained after transplantation of *wt* E-cadherin expressing MDA-MB-435S cells showed membranous E-cadherin staining in 20% of the neoplastic cells. (**C**) E-cadherin expression was detectable only in 5% of the neoplastic cells of tumors induced after injection of *del 8* E-cadherin expressing MDA-MB-435S cells. In contrast, much higher positivity was detectable in tumors with *del 9* E-cadherin mutation (**D**) and *p8* E-cadherin mutation (**E**). (**F**) A representative lung metastasis of an animal transplanted with *del 9* E-cadherin expressing MDA-MB-435S cells is shown. The neoplastic cells showed no E-cadherin reactivity while alveolar cells revealed membranous E-cadherin staining (arrow). Original magnification: (A, C and E) ×300, (B) ×200, (D and F) ×400.

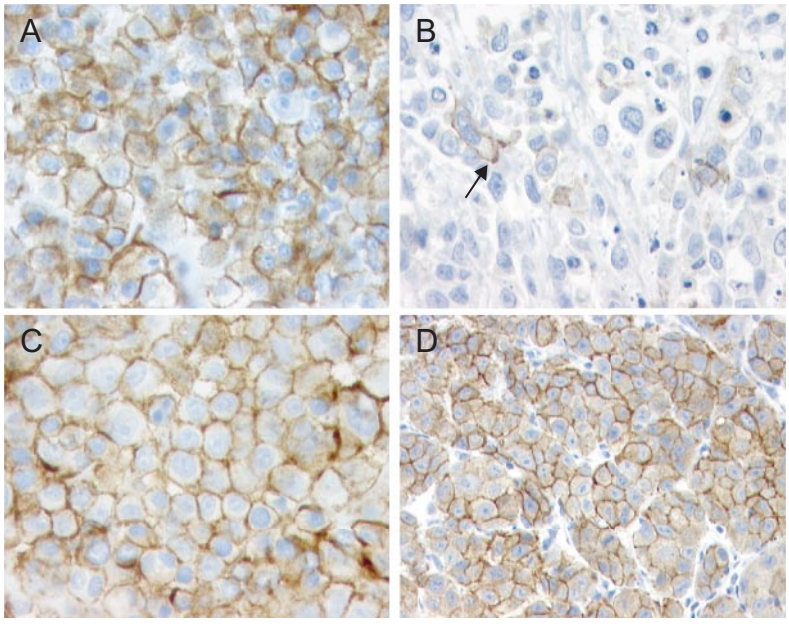

Fig. 4. Immunohistochemical study of *del 8* and *del 9* E-cadherin mutations in cell lines and corresponding xenograft tumors. (**A**) The cell line with *del 8* E-cadherin mutation showed a strong membranous positivity with *del 8*-specific antibody in the majority of the tumor cells. (**B**) In contrast, the mice tumors derived from this cell line revealed rare cells with membranous expression (arrow). The cell line with *del 9* E-cadherin (**C**) and the tumor derived from this cell line (**D**) showed a membranous staining in the majority of the tumor cells with the *del 9*-specific antibody. Original magnification: (A and D) ×400; (B and C) ×640.

Table I. Immunohistochemical analysis of E-cadherin in primary tumors

MDA-MB-435S-derived cell line	% positive neoplastic cells stained with antibody against the cytoplasmic E-cadherin domain or specific against mutant E-cadherin[a]		
	E-cadherin cyto. dom.	*del 8*-E-cad	*del 9*-E-cad
Parental	–		
	–		
wt	20		
	20		
del 8	5	5	–
	5	5	–
del 9	80	–	>90
	70	–	>90
p8	60		
	80		

cyto. dom., antibody against the cytoplasmic E-cadherin domain. *del 8*-E-cad, antibody against E-cadherin with deletion of exon 8. *del 9*-E-cad, antibody against E-cadherin with deletion of exon 9.
[a]Expression was evaluated in two mice per cell line.

and proliferation rate is that the dynamic of tumor growth may be different in tumors derived from *wt* and *del 8* E-cadherin expressing cells. Tumor growth induced by the highly proliferating *del 8* E-cadherin expressing cells may be limited by insufficient supply with nutrients, leading to extensive necrosis.

E-cadherin staining pattern of primary tumors is heterogeneous

Immunohistochemical analysis of E-cadherin expression revealed a heterogeneous staining pattern for all tumors. Tumors induced after inoculation of *wt* and *del 8* E-cadherin expressing MDA-MB-435S cells showed few E-cadherin positive tumor cells (20 or 5%, respectively). Tumors with *del 9* and *p8* E-cadherin mutations revealed much higher positivities (*del 9*: 70 and 80%; *p8*: 60 and 80%). To prove that E-cadherin expression was detectable after formalin fixation and paraffin embedding of the cells, cell blocks of the original cell lines were stained with mutation-specific E-cadherin antibodies. In accordance with previous results (25,33), both *del 8* and *del 9* deletion variants were 100% positive with the respective antibodies. While the cell blocks were positive for E-cadherin, tumors derived from *del 8* E-cadherin expressing cells showed occasional membranous staining. The data are in accordance with our previous observations that *del 8* E-cadherin was found to be localized perinuclear and only in punctuate areas at lateral membranous cell contacts in subconfluent MDA-MB-435S cells by laser scanning microscopy (29).

E-cadherin expression can be down-regulated by transcriptional repressors such as Snail, SIP1 and SLUG (13–16,35), by extracellular cleaving and shedding of E-cadherin mediated by matrix metalloproteinases (36,37) and by ubiquitination of the E-cadherin–catenin complex by Hakai, a c-Cbl-like protein (38). During apoptosis, cadherin-mediated cell–cell adhesion is disrupted by a mechanism that involves E-cadherin cleavage (39,40); the involved catalytic activities are caspases and metalloproteinases. Further observations point to an important role of the soluble tumor microenvironment for the presence of E-cadherin on tumor cells: plasmin has recently been shown to produce an E-cadherin fragment, thereby stimulating cancer cell invasion (41). However, transcriptional repression of E-cadherin as well as E-cadherin DNA methylation which frequently occurs in tumors (42), are unlikely to play a role in our model system, as the E-cadherin cDNA is expressed under the transcriptional control of the β-actin promoter, but not the native E-cadherin promoter.

In further experiments, it remains to be determined at which stage of tumor development E-cadherin is down regulated. As *wt* E-cadherin expressing cells induce small-size tumors, we hypothesize that down-regulation of E-cadherin occurs as a late event. The presence of the E-cadherin transgene was readily detectable by polymerase chain reaction in primary tumors derived from *wt* and *del 8* E-cadherin expressing MDA-MB-435S cells (data not shown). This result does not exclude, however, that rearrangements might have occurred in other regions of the E-cadherin cDNA. Further investigation of the mechanism of reduced E-cadherin transgene expression in some primary tumors is necessary before drawing final conclusions about the impact of mutant E-cadherin variants on tumor growth.

Down-regulation of E-cadherin in nude mouse tumors has been observed by other authors, for instance in Harvey-murine-sarcoma-virus-transformed Madin Darby canine kidney cells (MDCK-ras) which produce malignant (i.e. invasive and metastatic) tumors in nude mice (43). Primary tumors as well as large metastases were heterogeneous, showing E-cadherin-positive well differentiated epithelial structures and E-cadherin-negative undifferentiated areas. Metastasis-derived cell cultures contained both E-cadherin-positive and E-cadherin-negative MDCK-ras-e cells during early passages *in vitro*. During further culture, however, they regained the homogeneous E-cadherin-positive characteristic of the original MDCK-ras-e cell line. The authors hypothesize that this result points to the existence of host factors, which are able to down-regulate E-cadherin expression, and they hypothesize that this down-regulation plays a basic role in invasion.

Metastases are E-cadherin negative

Loss of E-cadherin-mediated adhesion may facilitate tumor cell detachment from the primary tumor and promote tumor cell dissemination. In the present study, all investigated cell lines induced lung metastases. All metastases were completely negative for E-cadherin. Since all primary tumors showed heterogenicity for E-cadherin expression, the data suggest that loss or down-regulation of E-cadherin expression may promote tumor cell detachment from the primary tumor and dissemination of malignant cells. The occurrence of metastases in a high percentage of cases show the dissociation between primary tumor growth and metastatic ability.

The role of E-cadherin in the process of metastasis formation of MDA-MB-435S carcinoma cells has been analysed previously (44). The mouse cDNA for E-cadherin was stably expressed in MDA-MB-435 carcinoma cells, and the altered cells were then injected into the mammary fat pads of nude mice, where they formed tumors, which spontaneously metastasized to the lungs. Expression of E-cadherin was inhibitory to metastasis formation. E-cadherin expression was detected throughout the primary tumors, but was completely absent in lung metastases. The authors concluded that induction of metastasis is detected when cells have lost epithelial characteristics.

The MDA-MB-435S cell line was accepted to be derived from a breast carcinoma and was widely used as a model in breast cancer reseach. Recent data indicate, however, that the MDA-MB-435S cell line might not originate from breast

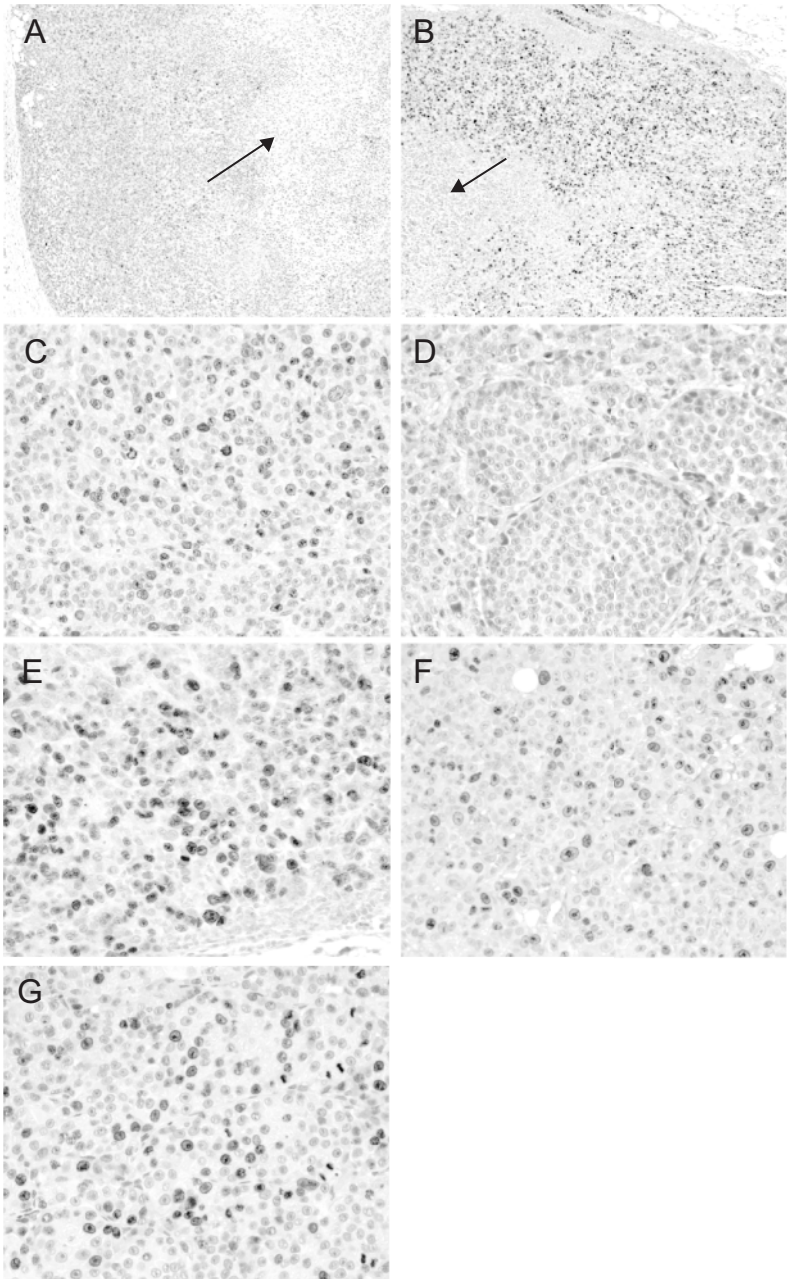

Fig. 5. Immunohistochemical study of MiB1 in primary tumors. MiB1 positive neoplastic cells in tumors derived from *wt* (**A**) or *del 8* (**B**) E-cadherin expressing cells are shown, the arrows point to necrotic areas. Higher magnification demonstrates differences in MiB1 positivity in parental MDA-MB-435S cells (**C**), *wt* E-cadherin expressing cells (**D**), *del 8* E-cadherin expressing cells (**E**), *del 9* E-cadherin expressing cells (**F**) and *p8* E-cadherin expressing cells (**G**). Original magnification: (A and B) ×50; (C–G) ×200.

cancer (45,46). Microarray data suggested that MDA-MB-435S cells might originate from an occult melanoma (45). To investigate this hypothesis further, two sublines of MDA-MB-435 (MDA-MB-435S and MDA-MB-435 HGF) were analyzed by reverse transcription–polymerase chain reaction and immunohistochemistry for the expression of genes characteristic of breast cells or melanocytes (46). The expression of breast genes was not detected, while melanocytic genes were expressed. These results provide further evidence that MDA-MB-435S cells might be of melanoma origin.

Several studies show that E-cadherin function is critical in the induction and maintenance of cell polarity and differentiation, and its functional or transcriptional loss is associated with an invasive and poorly differentiated phenotype (47,48). In the present study, we demonstrate that E-cadherin mutations impair the tumor-suppressive function of E-cadherin and/or alter the dynamics of tumor development in a SCID mouse model. The tumors showed heterogeneous E-cadherin staining patterns, which indicates a loss or down-regulation of E-cadherin during tumor development. Furthermore, loss of E-cadherin-mediated cell adhesion may promote tumor cell dissemination, as suggested in this study, where the E-cadherin staining was absent in lung metastases.

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