E-Cadherin-mediated Cell–Cell Adhesion Prevents Invasiveness of Human Carcinoma Cells

Uwe H. Frixen, Jürgen Behrens, Martin Sachs, Gertrud Eberle, Beate Voss, Angelika Warda, Dorothea Lötcher and Walter Birchmeier
Institut für Zellbiologie (Tumorfororschung), Essen Medical School, 4300 Essen 1, Germany

Abstract. The ability of carcinomas to invade and to metastasize largely depends on the degree of epithelial differentiation within the tumors, i.e., poorly differentiated being more invasive than well-differentiated carcinomas. Here we confirmed this correlation by examining various human cell lines derived from bladder, breast, lung, and pancreas carcinomas. We found that carcinoma cell lines with an epithelioid phenotype were noninvasive and expressed the epithelial-specific cell–cell adhesion molecule E-cadherin (also known as Arc-I, uvomorulin, and cell-CAM 120/80), as visualized by immunofluorescence microscopy and by Western and Northern blotting, whereas carcinoma cell lines with a fibroblastoid phenotype were invasive and had lost E-cadherin expression. Invasiveness of these latter cells could be prevented by transfection with E-cadherin cDNA and was again induced by treatment of the transfected cells with anti-E-cadherin mAbs. These findings indicate that the selective loss of E-cadherin expression can generate dedifferentiation and invasiveness of human carcinoma cells, and they suggest further that E-cadherin acts as an invasion suppressor.

Over 90% of the human tumors are carcinomas; in these, transformed epithelial cells grow in an uncontrolled fashion, break through the basement membrane, and invade the underlying mesenchyme. Carcinomas can be subdivided by morphological and functional criteria: (a) well-differentiated carcinomas largely retain epithelial tissue structures, they show well-developed intercellular junctions, and they are generally weakly invasive, and (b) poorly differentiated carcinomas are characterized by an amorphous tissue structure, they have fewer cell–to-cell junctions, and they are more invasive (Weinstein et al., 1976; Gabbert et al., 1985). It has also been shown that the state of differentiation and the degree of invasiveness of carcinomas can determine cancer prognosis. For instance, 80% of patients with well-differentiated colorectal carcinomas survive five and more years, in contrast to only 25% of patients with diagnosis of poorly differentiated colorectal carcinomas (Morson and Dawson, 1979).

These morphological and functional characteristics of carcinomas have been recognized years ago; the underlying molecular basis, however, is only presently being investigated. Various oncogenes have been found to be implicated in the genesis of human carcinomas, e.g., the Ki-ras oncogene (Bos et al., 1987; Forrester et al., 1987; Almoguera et al., 1988), and the HER-2/neu protooncogene (Slamon et al., 1987, 1989). Various tumor suppressor genes are mutated or deleted in human carcinomas, e.g., the retinoblastoma susceptibility gene (Harbour et al., 1988; Lee et al., 1988; Bookstein et al., 1990), the Wilms tumor gene (Habor et al., 1990; Gessler et al., 1990), the p53 gene (Takahashi et al., 1989; Nigro et al., 1989; Tsai et al., 1990), the deleted in colon carcinomas (DCC) gene (Fearon et al., 1990), a gene on chromosome 5q (Bodmer et al., 1987; Leppert et al., 1987), and a gene (or genes) on chromosome 3p (Kok et al., 1987; Zbar et al., 1987; Kovacs et al., 1988).

Keratins and desmosomal proteins allow the identification and classification of epithelial tumors and can also be used for tracing back the origin of metastatic carcinomas (Osborn et al., 1977; Gabbianelli et al., 1981; Osborn and Weber, 1983; Cooper et al., 1985; Moll et al., 1986). Changes in the keratin expression pattern which parallel dedifferentiation have recently been observed in bladder carcinomas (Moll et al., 1988). The carcinoembryonic antigen (CEA) has been found to be a valuable indicator of carcinomas of the gastrointestinal tract (Mentges, 1987; Benchimol et al., 1989; Daneker et al., 1989), the expression of urokinase and its receptor is restricted to dedifferentiated colon carcinoma cell lines (Boyd et al., 1988), and the estrogen receptor represents a useful marker for differentiated breast carcinomas (Engel and Young, 1978; Sluyser, 1990).

Our laboratory has studied epithelial differentiation and invasion with respect to the expression and function of the epithelium-specific cell–cell adhesion molecule E-cadherin, which various investigators have differently named Arc-I (Imhof et al., 1983; Behrens et al., 1985), uvomorulin (Hyafil et al., 1981; Vestweber and Kemler, 1984), L-CAM (Gallin et al., 1983), E-cadherin (Shirayoshi et al., 1983), and cell-CAM 120/80 (Damsky et al., 1987). We have demonstrated that nontransformed MDCK epithelial cells

1. Abbreviations used in this paper: CEA, carcinoembryonic antigen; DCC, deleted in colon carcinomas; DFKZ, German Cancer Research Center.
acquire invasive properties when intercellular adhesion is specifically inhibited by the addition of antibodies against E-cadherin; the separated cells then assume a fibroblast-like, i.e., dedifferentiated morphology, and invade collagen gels and embryonal heart tissue. Furthermore, MDCK cells transformed with Harvey and Moloney sarcoma viruses were found to be constitutively fibroblast-like and invasive, and they do not express E-cadherin. These data suggested that the loss of adhesive function of E-cadherin is a critical step in the promotion of epithelial cells to a dedifferentiated and invasive, i.e., malignant stage (Behrens et al., 1985, 1989).

The cell-adhesion molecule E-cadherin represents a 120-kD cell surface glycoprotein, of which extracellularly an 80-kD soluble tryptic fragment can be released in the presence of Ca++ (Hyafil et al., 1981; Damsky et al., 1983; Gallin et al., 1983; Peyrieras et al., 1983). In early mouse development E-cadherin functions as an adhesion component during compaction of blastomeres (Hyafil et al., 1981; Damsky et al., 1983; Shirayoshi et al., 1983; Vestweber and Kemler, 1984), at later stages it is confined to epithelia originating from ecto-, meso-, and endodermal tissue (Edelman et al., 1983; Damsky et al., 1983; Imhof et al., 1983; Vestweber and Kemler, 1984; Behrens et al., 1985). In the epithelium of the small intestine, E-cadherin is enriched in the adherens junctions, in other epithelia it is present at the lateral cell surfaces (Damsky et al., 1983; Boller et al., 1985; Behrens et al., 1985). The full cDNA of the mouse molecule has recently been cloned; it codes for a signal peptide at the NH2-terminus, a large extracellular domain with four repeats, a transmembrane domain, and a small cytoplasmic domain (Nagafuchi et al., 1987; Ringwald et al., 1987). After transfection of the cDNA into fibroblasts, functional Ca++-dependent contacts between the cells could be generated (Nagafuchi et al., 1987; Mege et al., 1988; Ozawa et al., 1989). E-cadherin belongs to a gene family; the closest relatives are N-cadherin (expressed in neural and muscle cells, Hatta et al., 1985, 1988) and P-cadherin (originally identified in human carcinoma cells). A further variant of human cadherin with four repeats, a transmembrane domain, and a short cytoplasmic domain could be isolated (Nagafuchi et al., 1989). Tryptic digests of the cells (Behrens et al., 1989) were chromatographed on an affinity column prepared by coupling 40 mg of the IgG fraction of the antisera to 5 ml CNBr-Sepharose. The 80-kD fragment could then specifically be eluted with 5 mM EDTA, 500 mM NaCl, 50 mM Tris-Cl, pH 8.5, and concentrated by acetone precipitation at -70°C. From 1 g of wet cells 1.5 µg of 80-kD tryptic fragment of human E-cadherin could be isolated.

For the production of mAbs, BALB/c mice were immunized with the antigen (3 µg per animal and immunization) using the ABM adjuvans system (Zymed Laboratories Inc., South San Francisco, CA). Spleen cells from immunized mice were fused with P3-X63-Ag 8.653 mouse myeloma cells (Kearney et al., 1979). The resulting hybridomas were cultured in multiwell tissue culture plates.

**Screening of mAbs**

Hybridoma supernatants were screened in an enzyme-linked immunoassay using 96-well microtiter plates (Nunc, Roskilde, Denmark) which were coated with 75 ng/well of the tryptic fragment of human E-cadherin. Antibodies bound to the solid-phase antigen were detected using peroxidase-coupled goat anti-mouse IgG antibodies (Jackson ImmunoResearch Laboratories, Avondale, PA). Positive hybridoma supernatants were rescreened by immunofluorescence on human A-431 carcinoma cells and on frozen sections of human small intestine following fixation with 3% formaldehyde and treatment with 0.5% Triton X-100, and by Western blot analysis of the 80-kD fragment and of total A-431 cell extracts. For preparation of these extracts, freshly scraped A-431 cells were lysed for 5 min at 96°C with 2% SDS, 5% 2-mercaptoethanol in L-CAM assay buffer (Cunningham et al., 1984) followed by centrifugation at 100,000 g at 12°C (Behrens et al., 1989). Antibody isotypes were determined using a mouse monoclonal isotyping kit (Serotec, Oxford, England).

**Analysis of E-cadherin in the Various Human Carcinoma Cell Lines using Immunological Methods**

For the immunofluorescence of E-cadherin in tissue culture cells, 100,000 g hybridoma supernatants or Protein A-purified antibodies (eluted from Protein A-Sepharose with 50 mM ethanolamine, pH 11) were used. Immunofluorescence of cytokeratins was carried out according to Sun and Green (1978) using broad-spectrum antibodies (Jackson ImmunoResearch Laboratories and Canom Labor Service, Wiesbaden, Germany). For comparison cell lines in Western blot experiments, 50 µg total cell protein (extracted with SDS-buffer, see above) were loaded per gel lane. For quantitative antibody binding, human carcinoma cells were cultured overnight in 96-well flexible microtiter plates (5 x 104 cells/well), fixed with 3% formaldehyde, and permeabilized with Triton X-100. After 1-h incubation with...
hybridoma supernatant (which was preabsorbed over fixed and permeabilized MRC-5 human lung fibroblasts for 1 h at room temperature) cells were washed and incubated with goat anti-mouse IgG (Jackson Immuno Research Laboratories Inc., Avondale, PA) labeled with 125I using the lactoperoxidase method (2 × 10⁷ cpm/μg; 5 × 10⁵ cpm/well). Wells were then cut off and radioactivity of triplicate experiments was determined in a gamma counter.

Northern Blot Analysis

To obtain a human E-cadherin cDNA-probe, a human liver cDNA library (in the plasmid expression vector pUEX1; a gift of U. Loggen, Essen, Germany) was screened. Out of 1.6 × 10⁵ clones, HC6-1 was picked and the insert was subcloned into M13 phages and sequenced in both directions by the dideoxy method using T7 DNA polymerase (Pharmacia Fine Chemicals, Piscataway, NJ).

For preparation of RNA, cells were washed with PBS, scraped off the plates with a rubber policeman, and lysed on ice in 1% NP40, 140 mM NaCl, 1.5 mM MgCl₂, 100 μg/ml heated salmon sperm DNA. Filters were washed at room temperature with 2× SSC (2 × 10⁻² M NaCl, 1.5 mM MgCl₂, 100 U RNasin/ml, 10 mM Tris-C1, pH 8.0). After centrifugation at 12,000 rpm in a rotor (model JA20; Beckman Instruments, Inc., Palo Alto, CA), the supernatant was extracted at 65°C with acid phenol containing 1% SDS, 10 mM EDTA, followed by extraction at room temperature with Tris-buffered phenol, phenol/chloroform, chloroform, and precipitation with ethanol at -20°C. Poly A⁺-RNA was obtained by affinity chromatography of total RNA on oligo (dt)-cellulose using the spin column method (Pharmacia Fine Chemicals). RNA was electrophoresed on glyoxal gels (Maniatis et al., 1982) followed by blotting on Hybend-N membranes (Amersham Corp., Arlington Heights, IL). Hybridization of the blots was carried out with nick-translated cDNA-probes overnight at 64°C in 5× SSC, 5× Denhardt's solution, 10% dextran sulfate (Pharmacia Fine Chemicals), 0.5% SDS, 100 μg/ml heated salmon sperm DNA. Filters were washed at room temperature with 2× SSC (2 × 10⁻² M NaCl, 1.5 mM MgCl₂, 100 U RNasin/ml, 10 mM Tris-C1, pH 8.0) followed by autoradiography with intensifying screens at -70°C.

Transfection of Mouse E-cadherin cDNA into Dedifferentiated Human Carcinoma Cells

A subclone of dedifferentiated human breast carcinoma cells (i.e., MDA-MB-453/S1) was transfected using the Ca⁺⁺-phosphate coprecipitation method with the full-length cDNA of mouse E-cadherin (plasmid pBATEM 2; E-cadherin under the control of the chicken β-actin promoter; a gift of A. Nagafuchi and M. Takeichi, Kyoto, Japan) together with a plasmid for neomycin resistance. G 418-resistant clones which exhibited an epithelioid morphology in tissue culture, whereas most cell lines obtained from the respective poorly differentiated tumors expressed fibroblasticoid morphology (data not shown, cf. also Behrens et al., 1989). In the immunofluorescence of cytokeratins using a broad-spectrum antiserum that recognizes all keratin types, some enrichment was seen in the area of the junctional complex (data not shown, cf. also Behrens et al., 1985).

A partial E-cadherin cDNA clone, HC6-1, was isolated from a human liver plasmid cDNA library, and the insert was sequenced. It encompasses 386 bp, which are homologous to position 865-1251 of the full-size mouse E-cadherin cDNA (Nagafuchi et al., 1987), and the first 142 bp are identical to the 3' end of the published sequence of another partial human E-cadherin cDNA clone (Mansouri et al., 1988). The sequence of HC6-1 has the number X 52279 in the EMBL data bank.

Expression of E-cadherin in Differentiated and Dedifferentiated Human Carcinoma Cell Lines

A variety of differentiated and dedifferentiated human carcinoma cell lines were obtained from the tumor cell bank of the German Cancer Research Center (DKFZ) and from the American Type Culture Collection (ATCC). We observed that cell lines isolated from different organs expressed a fibroblasticoid phenotype in tissue culture, whereas most cell lines obtained from the respective poorly differentiated tumors expressed an epithelioid morphology (data not shown, cf. also Behrens et al., 1985).

By applying the new mAbs and the human cDNA probe we found that the differentiated (epithelioid) carcinoma cell lines expressed E-cadherin, whereas the dedifferentiated (fibroblastoid) lines were usually negative. For instance, when tested by immunofluorescence with antibody 6F9, the differentiated bladder carcinoma cell line RT4 expressed E-cadherin in the areas of cell-cell contact (Fig. 2, a and b), whereas the dedifferentiated bladder carcinoma cell line
Table I. Characterization of the Human Carcinoma Cell Lines

| Cell line     | Characteristics related to differentiation                                                                 | Morphology of cultured cells* |
|---------------|------------------------------------------------------------------------------------------------------------|-------------------------------|
| **Bladder**   |                                                                                                            |                               |
| RT4           | derived from a well-differentiated carcinoma (Rigby and Franks, 1970)                                       | E                             |
| RT112         | derived from a well-differentiated carcinoma (Steele et al., 1983)                                         | E                             |
| EJ28          | derived from an anaplastic carcinoma (Hastings and Franks, 1983)                                          | F                             |
| **Colon**     |                                                                                                            |                               |
| CX-1          | well-differentiated cell line (Danecker et al., 1989)                                                       | E                             |
| WiDr          | derived from an adenocarcinoma, differentiated cell line (Noguchi et al., 1979)                              | E                             |
| HCT116        | epithelioid cell line in tissue culture (our finding; compare with Boyd et al., 1988)                       | E                             |
| SW948         | derived from an undifferentiated adenocarcinoma, differentiated in culture (Leibowitz et al., 1976)         | E                             |
| COLO205       | derived from an anaplastic adenocarcinoma, differentiated in tissue culture (Semple et al., 1978)           | E/Sph+                        |
| SW620         | dedifferentiated cell line (Leibowitz et al., 1976)                                                         | Sph+                          |
| **Breast**    |                                                                                                            |                               |
| MCF-7         | characteristics of differentiated mammary epithelium; estrogen receptor-positive‡ (Soule et al., 1973; Engel and Young, 1978) | E                             |
| MDA-MB-361    | estrogen-receptor-positive cell line (Engel and Young, 1978)                                               | E                             |
| BT-549        | derived from an invasive ductal tumor (ATCC)                                                                | F/Eli                         |
| MDA-MB-231    | derived from a poorly differentiated carcinoma, spindle-shaped in culture; estrogen receptor-negative (Cailleau et al., 1974; Engel and Young, 1978) | F                             |
| MDA-MB-435S   | spindle-shaped cell line (Cailleau et al., 1978)                                                            | F                             |
| MDA-MB-436    | spindle-shaped cell line (Cailleau et al., 1978)                                                            | F                             |
| **Lung**      |                                                                                                            |                               |
| LX-1          | derived from a human tumor which was characterized as a moderately well-differentiated adenocarcinoma after passage in nude mice (H. Lührke, Heidelberg, personal communication) | E                             |
| A-427         | epithelial-like cell line, many cells with bizarre shapes (Giard et al., 1973)                               | E/F/Eli                       |
| A-549         | some characteristics of type II alveolar epithelial cells; vimentin-positive (Lieber et al., 1976; Blobel et al., 1984) | F/E/Eli                       |
| LXF289        | derived from a moderately well-differentiated adenocarcinoma; dedifferentiated morphology in culture (H. Lührke, Heidelberg, personal communication) | F                             |
| SK-MES-1      | cell line from a squamous carcinoma; vimentin-positive (Fogh and Trempe, 1975; Blobel et al., 1984)          | F                             |
| **Pancreas**  |                                                                                                            |                               |
| Capan-1       | well-differentiated adenocarcinoma cells (Fogh et al., 1977)                                               | E                             |
| Capan-2       | adenocarcinoma cell line (Fogh et al., 1977)                                                                | E                             |
| DAN-G         | derived from a well-differentiated carcinoma (H. Lührke, Heidelberg, personal communication)             | E                             |
| Hs 766T       | derived from a lymph node metastasis (Owens et al., 1976)                                                   | F/Eli                         |
| MIA PaCa-2    | derived from an undifferentiated carcinoma (Yunis et al., 1977)                                             | F                             |

* Cells were grouped according to their morphology in cell culture as epithelioid (E) or fibroblastoid (F).
† Sph, cells showed a spherical morphology.
‡ The estrogen receptor is a widely used marker for differentiated mammary carcinomas (Engel and Young, 1978).
§ Cell lines with intermediate morphology, whereby the first letter indicates the dominant form.

EJ28 did not show any fluorescence (Fig. 2, c and d). Similarly, the differentiated breast (MCF-7; Fig. 2, e and f), lung (LX-1; Fig. 3, a and b) and pancreas (DAN-G; Fig. 3, e and f) carcinoma cell lines expressed E-cadherin whereas the corresponding dedifferentiated breast (MDA-MB-435S; Fig. 2, g and h), lung (LXF289; Fig. 3, c and d), and pancreas (MIA PaCa-2; Fig. 3, g and h) carcinoma cell lines were negative.

The total amount of E-cadherin in these cell lines, as observed by Western blotting, largely corresponded with the immunofluorescence data. For instance, extracts of the differentiated bladder and breast carcinoma cell lines were positive for the 120-kD polypeptide, whereas the dedifferentiated bladder and breast carcinoma cell lines were negative (Fig. 4). These differences were also seen with the second antibody 15C12 (data not shown). Northern blots revealed similar differences in the E-cadherin mRNA content. For example, the differentiated bladder, breast, lung, and pancreas carcinoma cell lines expressed E-cadherin-mRNA well, whereas the corresponding dedifferentiated carcinoma cell lines did not show this message (Fig. 5). Cell lines with intermediate phenotypes (e.g., A-427 and Hs 766T) expressed small amounts of mRNA.

We also examined the human carcinoma cell lines (in par-
Figure 2. Immunofluorescence staining for E-cadherin in human bladder and breast carcinoma cell lines. The new mAb 6F9 was used in this experiment. (a and b) Differentiated bladder carcinoma cell line RT4; (c and d) dedifferentiated bladder carcinoma line EJ28; (e and f) differentiated breast carcinoma cell line MCF-7; and (g and h) dedifferentiated breast carcinoma line MDA-MB-435S. Apparently, the differentiated cell lines express E-cadherin at their cell-cell contact sites, whereas the dedifferentiated lines are negative. Bar, 20 μm.
Figure 3. Immunofluorescence staining for E-cadherin in human lung and pancreas carcinoma cell lines. mAb 6F9 was used. (a and b) Differentiated lung carcinoma cell line LX-1; (c and d) dedifferentiated lung carcinoma line LXF289; (e and f) differentiated pancreas carcinoma cell line DAN-G; and (g and h) the dedifferentiated pancreas carcinoma cell line MIA PaCa-2. The differentiated lines express E-cadherin at the cell-cell contact sites, whereas the dedifferentiated lines are negative. Bar, 20 μm.
E-cadherin. (lanes a and b) Extracts of the differentiated bladder carcinoma cell lines RT112 and RT4; (lane c) of the dedifferentiated bladder carcinoma line EJ28; (lane d) of the differentiated breast carcinoma cell lines RT112 and RT4; (lane c) of the dedifferentiated breast carcinoma lines MDA-MB-231, -435S, and -436 were analyzed using mAb 6F9; (lane h) is a control extract prepared by our probe. We also tested whether the lack of expression of E-cadherin in dedifferentiated cell lines might be because of hypermethylation of the gene (cf., Feinberg and Vogelstein, 1983; Feinberg et al., 1988). In contrast, we found that the dedifferentiated cell lines rather exhibited a lower degree of methylation in the probed genomic region, as seen by new bands created by digestion with the enzyme HpaII (data not shown).

Correlation between E-cadherin Expression and Invasiveness In Vitro

We then examined the various human carcinoma cell lines for invasiveness in the collagen assay (Behrens et al., 1989) and found a striking inverse correlation with the degree of E-cadherin expression. Thus, E-cadherin positive cell lines were largely noninvasive for collagen gels, whereas the E-cadherin-negative lines did penetrate into the gel (Fig. 6). Clear-cut examples are the bladder carcinoma cell lines RT112, RT4, and EJ28; in the cases of the breast, lung, and pancreas carcinoma cell lines, a gradual shift from the E-cadherin-expressing noninvasive state to the nonexpressing invasive state was observed. The differentiated colon carcinoma cell lines were all noninvasive; the fact that a largely dedifferentiated cell line (SW620) did not invade might be because of the predominantly spherical morphology of the cells (see also Table 1).

Prevention of the Invasiveness of Human Breast Carcinoma Cells by Transfection with E-cadherin cDNA

Mouse E-cadherin cDNA was transfected into human breast carcinoma cells MDA-MB-435S/1 (see Materials and Methods), and clones expressing various amounts of mouse E-cadherin were observed. Of these, three stable epithelioid clones were finally established: clone cad-B1 expressed mouse E-cadherin well at the cell-cell contacts, as shown by staining with the mAb DECMA-1 (Fig. 7, a and b), whereas a control clone neo-B4 did not (Fig. 7, c and d). Cad-B1-cells were still negative for human E-cadherin, as tested with antibody 6F9 (Fig. 7 e; note that the two antibodies DECMA-1 and 6F9 react in a species-specific manner with the mouse and human molecule, respectively; compare Fig. 7, b, e, and f).

The transfected breast carcinoma cells were then examined for invasiveness in vitro. The E-cadherin-expressing clone cad-B1 was found to be five times less invasive for collagen gels than the control clone neo-B4 (compare the black columns in Fig. 8, a and b) or the parental cell line (Fig. 6). However, full invasiveness of the cad-B1 cells could be regenerated by dissociating them with antibody DECMA-1 (which specifically recognizes mouse E-cadherin, compare the checkered columns in Fig. 8, a and b). As a control, DECMA-1 also induced invasiveness of mouse carcinoma cells CSG 120/7 (Fig. 8 c). These data indicate that E-cadherin expression converts human breast carcinoma cells MDA-MB-435S from a dedifferentiated invasive to a differentiated noninvasive state. They thus complement the experiments in which dedifferentiated invasive epithelial cells were generated by disturbance of E-cadherin-mediated cell-cell adhesion with specific antibody treatment (Fig. 8 e; see also Behrens et al., 1989).

Discussion

In this study we examined the expression of the cell-cell adhesion molecule E-cadherin (which has also been named Arc-1, uvomorulin, and cell-CAM-120/80) in a variety of human carcinoma cell lines originating from different tissues. Particular emphasis was directed toward changes of E-cadherin expression which occur in the process of tumor dedifferentiation. We found that cell lines derived from well differentiated human carcinomas (which keep the epithelial phenotype in tissue culture, 14 cell lines were tested) generally express E-cadherin well, whereas the molecule is not detected in most cell lines from poorly differentiated carcinomas (which exhibit a fibroblastoid phenotype in tissue culture, nine cell lines were tested). As a correlate, E-cadherin-negative cell lines were found to be invasive for collagen gels, whereas E-cadherin-positive lines did generally not enter the extracellular matrix. Furthermore, the invasiveness of dedifferentiated breast carcinoma cells could be corrected by transfection with E-cadherin cDNA. These results show
that E-cadherin expression is a decisive indicator for differ-
entiation and invasiveness of human carcinoma cells, and
they suggest that E-cadherin should now thoroughly be ex-
amined for its possible role in preventing tumor cell invasion
in vivo.

**Specificity of Probes**

We have here used new mAbs and a cDNA-probe for ex-
amining E-cadherin expression in the various human carci-
noma cell lines. Since there exists a family of Ca²⁺-depen-
dent cell-adhesion molecules, how can we be sure that our
probes are specific for the epithelial form of the cadherins?
First, we do not need to consider N-cadherin here, since this
molecule exhibits a clearly different protease digestion pat-
tern and a grossly different tissue distribution (Hatta et al.,
1985; Volk and Geiger, 1986; Takeichi, 1988). Second, the
antigen used for immunization was prepared by affinity chro-
matography on a polyclonal anti-E-cadherin antibody (i.e.,
our anti-Arc-I), and antibodies seem not to crossreact be-
tween E- and P-cadherin (Behrens et al., 1985, 1989; Hatta
et al., 1985; Takeichi, 1988; Shimoyama et al., 1989a).
Third, our mAbs stain the epithelium of the human small
intestine and the cell contacts of MCF-7 human breast car-
cinoma cells, which exclusively express E- but not P-cad-
herin (Shimoyama et al., 1989a). The sequence of the hu-
mn cDNA-probe was found to be most closely related to
mouse E-cadherin and it is identical in its overlapping region
to a partial human E-cadherin cDNA (Nagafuchi et al.,

---

**Figure 5.** Northern blot analysis of various human carcinoma cell lines for the presence of E-cadherin mRNA. Electrophoresed poly A⁺
RNA was blotted onto Hybond filters and hybridized with the HC6-1 cDNA probe (which recognizes E-cadherin mRNA) and with an
actin cDNA probe (for the demonstration of the loading in the three experiments). (A) Bladder (lanes a–c) and breast (lanes d–g) carcinoma
cell lines: (lane a) RTI12; (lane b) RT4; (lane c) EJ28; (lane d) MCF-7; (lane e) MDA-MB-231; (lane f) MDA-MB-435S; and (lane g)
MDA-MB-436. (B) Lung (lanes a–d) and pancreas (lanes e–g) carcinoma cell lines: (lane a) LX-1; (lane b) A-427; (lane c) SK-MES-1;
(lane d) LXF289; (lane e) Capan-2 (lane f) DAN-G; and (lane g) Hs 766T. (lane h) Control mRNA from the bladder carcinoma cell
line RTI12. (C) Colon carcinoma cell lines: (lane a) CX-1; (lane b) HCT116; (lane c) SW620; (lane d) shows the absence of E-cadherin
mRNA in MRC-5 human fibroblasts; (lane e) is control mRNA from the bladder carcinoma cell line RTI12. Apparently, the differentiated
carcinoma cell lines express E-cadherin mRNA, whereas the dedifferentiated lines are negative. The intermediate lines (A-427 and Hs
766T) express a small amount of mRNA (cf. with Table I).
E-cadherin is a valuable marker of differentiated noninvasive human carcinoma cells

Previously, human MCF-7 breast carcinoma, human A-431 epidermoid carcinoma, and human JAR gestational choriocarcinoma cells (which are all of the differentiated type) were found to express E-cadherin (Damsky et al., 1983). No dedifferentiated carcinoma cell lines were examined in this early report. Recently, E-cadherin expression was studied in low and high metastatic variants of the murine ovarian carcinoma cell line OV2944. In analogy to our present findings, a highly metastatic subline expressed only small amounts, as compared with the high level found in weakly metastatic lines (Hashimoto et al., 1989). The cell lines we have used in the present study are of much greater variety of tissue origin, and they were particularly selected to belong to either the differentiated or dedifferentiated type. For instance, the bladder carcinoma cell lines RT112, RT4 (both differentiated), and EJ28 (dedifferentiated) were previously characterized for other epithelial markers; their cytokeratin expression pattern shows a good correlation with that of corresponding urothelial (transitional cell) carcinomas of the respective grade of malignancy (Moll et al., 1988). Furthermore, EJ28 cells metastasized from the bladder of nude mice whereas RT4 cells did not (Ahlering et al., 1987). These bladder carcinoma cell lines revealed a striking difference with respect to E-cadherin expression and corresponding invasiveness (Fig. 6). The breast carcinoma cell lines used were previously characterized as either hormone dependent (e.g., the differentiated line MCF-7, which expresses the estrogen receptor) or hormone independent (three dedifferentiated MDA-MB-lines, which do not express the estrogen receptor; Engel and Young, 1978). In addition, the MDA-MB-lines 231, 435S, and 436 synthesize vimentin and only low levels of keratins (Sommers et al., 1989), which is also consistent with their dedifferentiated state. Our study now shows that E-cadherin represents an additional differentiation marker of breast carcinoma cell lines which inversely correlates with invasiveness in vitro. In conclusion, E-cadherin thus functions as a differentiation marker for a variety of human carcinoma cells, i.e., from bladder, breast, lung, pancreas, and possibly other carcinomas, which is of more generality than other markers mentioned above.
Figure 7. Immunofluorescence staining of mouse E-cadherin in transfected human breast carcinoma cells MDA-MB-435S/1. (a, b, and e) Human breast carcinoma clone cad-B1 transfected with mouse E-cadherin cDNA; (c and d) human control clone neo-B4; and (f) mouse CSG 1207 carcinoma cells. The cells were stained with either mAb DECMA-1, which recognizes mouse E-cadherin (b, d, and f), or with antibody 6F9, which marks human E-cadherin (e). Note that the transfected clone cad-B1 expresses mouse E-cadherin at the cell-cell contacts. Bar, 20 $\mu$m.

E-cadherin expression of various human carcinomas was studied by others on tissue sections, and the molecule was found to be expressed in both differentiated and dedifferentiated tumors (Eidelman et al., 1989; Shimoyama et al., 1989a). However, much lower levels seem to be present in poorly differentiated carcinomas (see Fig. 7F in Shimoyama et al., 1989a). We have recently also examined the expression of E-cadherin on sections of various human carcinomas. We found a good correlation with the state of differentiation in the cases of ovarian carcinomas (Pfisterer et al., 1990), of lobular breast carcinomas (Frixen, U. H., R. Moll, and W. Birchmeier, manuscript in preparation), and of squamous
carcinomas of the head and neck (Schipper, J., U. H. Fri xen, K. Jahnke, and W. Birchmeier, manuscript in preparation). An exception to the rule are ductal breast carcinomas; here invasive forms largely retain epithelial characteristics and express E-cadherin.

**E-cadherin Triggers the Conversion between the Invasive and Noninvasive States of Carcinoma Cells**

Considerable effort has been made in our laboratory and in the one of F. Van Roy and M. Mareel, Ghent, Belgium, to prove experimentally that E-cadherin expression is actually causal for inducing differentiation and preventing invasiveness of epithelial cells. First, transfection with E-cadherin cDNA converted both dedifferentiated breast carcinoma and ras-transformed MDCK cells toward the noninvasive phenotype (this report; Mareel et al., 1990). Second, treatment of differentiated salivary gland carcinoma and MDCK epithelial cells with anti-E-cadherin antibodies induced invasiveness (Fig. 8c; Behrens et al., 1989). These combined data suggest that E-cadherin represents a kind of master molecule for regulating the maintenance of epithelial differentiation. However, other factors can also come into play; for instance, scatter factor induces invasiveness of MDCK epithelial cells without affecting E-cadherin expression (Weidner et al., 1990), suggesting that this factor acts via another mechanism.

**Control of E-cadherin Gene Expression**

We have observed that the relative amounts of E-cadherin protein and mRNA largely correspond to each other in the various carcinoma cell lines tested. When the E-cadherin gene was examined by Southern blotting, no deletion or gross rearrangement was yet detected in the nonexpressing cell lines. Thus, these results suggest that E-cadherin expression is largely controlled on the transcriptional level. To further examine the molecular mechanisms that regulate E-cadherin gene expression, the putative promoter of the E-cadherin gene has recently been isolated and characterized. A 270-bp genomic fragment (between -178 and -92) was found to be sufficient to induce a 20-fold higher CAT (chloramphenicol acetyltransferase) expression in epithelial cells than in fibroblasts, indicating that cis-regulatory elements control epithelial-specific E-cadherin expression (Behrens, J., and W. Birchmeier. 1990). J. Cell Biol. 111:157.

In human hepatocellular carcinomas a loss of heterozygosity on chromosome 16 was recently detected in 52% of the informative cases, and the common region of allele loss was localized between the positions 16q22.1 and 23.2 (Tsuda et al., 1990). The human E-cadherin gene is located at position 16q22.1 (Natt et al., 1989). Furthermore, loss of heterozygosity on chromosome 16 was much more frequent in poorly differentiated than in well-differentiated liver carcinomas. Thus, the E-cadherin gene is a good candidate for the tumor suppressor gene of chromosome 16 in hepatocellular carcinomas. Accordingly, the tumor suppressor gene "fat" of Drosophila has recently been identified as a cadherin-like molecule (C. Goodman, personal communication).

**Comparison with Other Components Involved in Invasion and Metastasis of Human Carcinomas**

Recently, the nm 23/awd gene product was identified by Steeg and collaborators, which is reduced in human melanomas, carcinomas, and sarcomas of high metastatic potential, and which is homologous to the developmentally regulated gene abnormal wing discs of Drosophila (Rosengard et al., 1989). Reintroduction of this gene (which encodes for a cytoplasmic protein) into highly metastatic cells led to a correction of the metastatic phenotype. The DCC gene, which is located on human chromosome 18q, was found to be affected in 70% of colorectal tumors (Fearon et al., 1990). This gene codes for a putative cell-adhesion molecule of the N-CAM type, i.e., belongs to the immunoglobulin superfamily. The tumor marker CEA, which also belongs to the immunoglobulin superfamily, was found to function as a cell–cell adhesion molecule in colon adenocarcinoma cells (Benchimol et al., 1989). It is therefore likely that both cytoplasmic and cell surface proteins influence invasion and metastasis. The components discussed above (nm 23, DCC, 16q22.1 - 23.2, E-cadherin, fat) seem to promote the pro-
cess when lost, i.e., might represent true invasion and metastasis suppressors.

We would like to thank H. Löhrke, tumor bank of the DKFZ, Heidelberg, Germany, for providing human carcinoma cell lines, A Nagafuchi and M. Takeichi, Kyoto, Japan, for the generous gift of the plasmid pBATEM 2, R. Lelekakis, Germany, for antibody DECMA-1, Anja Cremers, Inge Spratte, and Astrid Braun-Saure for excellent technical assistance, and B. Lelekakis for excellent secretarial work.

This work was supported by the Deutsche Krebshilfe and the Bundesministerium für Forschung und Technologie.

Received for publication 22 October 1990 and in revised form 2 January 1991.

References

Ahlering, T. E., L. Dubeau, and P. A. Jones. 1987. A new in vivo model to study invasion and metastasis of human bladder carcinoma. Cancer Res. 47:6660-6665.

Almoguera, C., D. Shibata, K. Forrester, J. Martin, N. Arzheim, and M. Peruchio. 1988. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. Cell. 53:549-554.

Behrens, J., W. Birchmeier, S. L. Goodman, and B. A. Imhof. 1985. Dissociation of Madin-Darby canine kidney epithelial cells by the monoclonal antibody anti-Arc-1: mechanistic aspects and identification of the antigen as a component related to uvomorulin. J. Cell. Biol. 101:1307-1315.

Bodies, J., A. Pronstka, W. Cavenee, R. L. Neve, S. H. Orkin, and G. A. P. Bruns. 1990. Homozygous deletion in Wilms’s tumor of a zinc finger gene identified by chromosome jumping. Nature (Land.). 343:774-777.

Forrester, K., D. A. Almoguera, K. Han, W. E. Grizzle, and M. Peruchio. 1987. Detection of high incidence of K-ras genes during human colon tumorigenesis. Nature (Land.). 327:298-303.

Gabbert, H. R., Wagner, R. Moll, and C.-D. Gerharz. 1985. Tumor dedifferentiation: an important step in tumor invasion. Clin. Exp. Metastasis. 3:257-279.

Gabbi, G., Y. Kapanic, P. Barazzone, and W. W. Franke. 1981. Immunohistochemical identification of intermediate-sized filaments in human neoplastic cells: a diagnostic aid for the surgical pathologist. Am. J. Pathol. 104:206-216.

Gall, W. F., C. J. Bayley, J. Bodmer, H. J. R. Bussey, A. Ellis, P. Gorman, and D. E. Housman. 1989. Relationship between extracellular matrix interactions and degree of tumorigenicity of human prostate carcinoma cells by replacing target antigen in animal pattern formation. Proc. Natl. Acad. Sci. USA. 86:7289-7293.

Habre, D. A., A. J. Buckler, T. Glaser, K. M. Call, P. J. Cloth, R. L. Sohn, E. C. Douglass, and D. E. Housman. 1990. An internal deletion within an 11p13 zinc finger gene contributes to the development of Wilms’s tumor. Cell. 61:1257-1269.

Harbour, J. W., S.-L. Lai, J. Whang-Peng, A. F. Gazdar, J. D. Minna, and F. W. Kaye. 1988. Abnormalities in structure and expression of the retinoblastoma gene in SCLC. Science (Wash. DC). 241:353-357.

Hashimoto, M., O. Niwa, Y. Nitta, M. Takeichi, and K. Yokoro. 1989. Unstable expression of E-cadherin adhesion molecules in metastatic ovarian tumor cell lines. Jpn. J. Cancer Res. 80:439-453.

Hastings, R. J., and L. M. Franks. 1983. Cellular heterogeneity in a tissue culture cell line derived from a human bladder carcinoma. Br. J. Cancer. 47:233-244.

Hanaoka, K., T. S. Okada, and M. Takeichi. 1985. A monoclonal antibody disrupting calcium-dependent cell-cell adhesion of brain tissues: possible role of its target antigen in animal pattern formation. Proc. Natl. Acad. Sci. USA. 82:2789-2793.

Hata, A., V. Nose, A. Nagafuchi, and M. Takeichi. 1988. Cloning and expression of CDNA encoding a neural calcium-dependent cell adhesion molecule: its identity in the cadherin gene family. J. Cell. Biol. 106:873-881.

Hydfall, C., and F. Jacob. 1981. Cell-cell interactions in early embryogenesis: a molecular analysis of the role of calcium. Cell. 26:447-454.

Imhof, B. A., H. P. Vollmers, S. L. Goodman, and W. Birchmeier. 1983. Cell-cell interaction and polarity of epithelial cells: specific perturbation using a monoclonal antibody. Cell. 35:667-675.

Kearney, J. F., A. Rachruch, B. Liesegang, and K. Rajewsky. 1979. A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. J. Immunol. 123:1548-1550.

Klimpfinger, M., A. Beham, and H. Denk. 1987. The pathological findings in colorectal carcinomas and discussion of their significance regarding optimal tumor therapy. Wien. Klin. Wochenschr. 99:488-493.

Knowles, M. A., and L. M. Franks. 1977. Stages in neoplastic transformation of adult epithelial cells by 7,12-dimethylbenz(a)anthracene in vitro. Cancer Res. 37:3917-3924.

Koch, P. J., M. Walsh, M. Schmelz, M. D. Goldschmidt, R. Zimbelman, and W. Franke. 1990. Identification of desmoglein, a constitutive desmoplakin glycoprotein as a member of the cadherin family of cell adhesion molecules. Eur. J. Cell Biol. 53:1-12.

Kok, K., J. Oisinga, B. Carrit, M. B. Davis, A. H. Van Der Hout, A. Y. Van Der Veen, R. M. Landsmeer, E. L. M. De Leij, H. H. Berends, P. E. Post, S. Poppema, and C. M. Buys. 1987. Deletion of DNA sequence at the chromosomal region 3p21 in all major types of lung cancer. Nature (Land.). 330:578-581.

Kovacs, G., R. Erdlindsson, P. Boldog, S. Ingvarsson, R. Muller-Bredtin, G. Habermann, and I. Siumegi. 1988. Consistent chromosome 5p deletion and loss of heterozygosity in renal cell carcinoma. Proc. Natl. Acad. Sci. USA. 85:1571-1575.

Lee, E. Y.-H., P. Lee, H. To, J.-Y. Shew, R. Bookstein, P. Scully, and W.-H. Lee. 1988. Inactivation of the retinoblastoma susceptibility gene in human...
breast cancers. Science (Wash. DC). 241:218-221.
Leibowitz, A., J. C. Stinson, W. B. McCombs III, C. E. McCoy, K. C. Mazur, and N. D. Mably. 1976. Classification of human colorectal adenocarcinoma cell lines. Cancer Res. 36:4562-4569.
Leeper, S. M., D. Dobbs, P. Scallenher, P. O'Connell, Y. Nakamura, D. Stauffer, S. Woodward, R. Burt, J. Hughes, E. Gardner, M. Lathrop, J. Wasmuth, J.-M. Lalouel, and R. White. 1987. The gene for familial polyposis coli maps to the long arm of chromosome 5. Science (Wash. DC). 238:141-145.
Lieber, M., B. Smith, A. Szakal, W. Nelson-Rces, and G. Todaro. 1976. A continuous tumor-cell line from a human lung carcinoma with properties of type II alveolar epithelial cells. Int. J. Cancer. 17:62-70.
Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Mansouri, A., N. Sputt, P. N. Goodfellow, and R. Kemler. 1988. Characterization and chromosomal localization of the gene encoding the human cell adhesion molecule uvomorulin. Differentiation. 38:67-71.
Mareel, M., F. M. Van Roy, and P. De Batelier. 1990. The invasive phenotypes. Cancer and Metastasis Rev. 9:45-52.
Mege, R.-M., F. Matsuzaki, W. J. Gallin, J. I. Goldberg, B. A. Cunningham, and G. M. Edelman. 1988. Construction of epithelial sheets by transfection of mouse sarcoma cells with CDNA's for chicken cell adhesion molecules. Proc. Natl. Acad. Sci. USA. 85:7274-7278.
Meneges, B. 1987. Kolonkarzinom. Prüfverfahren CEU, Tumordifferenzierung und Prognose. Dtsch. Med. Wochr. 112:1245-1249.
Miyatani, S., K. Shimamura, M. Hatta, A. Nagafuchi, A. Nose, M. Matsunaga, K. Hatta, and M. Takeichi. 1989. Neural cadherin: role in selective cell-cell adhesion. Science (Wash. DC). 245:631-635.
Moll, R. 1986. Epithelial tumor markers. Verh. Dtsch. Ges. Pathol. 70:28-50.
Moll, R., P. Cowin, H.-P. Kapprell, and W. W. Franke. 1986. Biology of disease. Desmosomal proteins: new markers for identification and classification of tumors. Lab. Invest. 54:4-25.
Moll, R., R. A. Aicher, G. Ebert, T. Balsiger, M. Itensohn, and W. W. Franke. 1988. Cytokeratins in normal and malignant transitional epithelium. Maintenance of expression of urothelial differentiation features in transitional cell carcinomas and bladder carcinoma cell culture lines. Am. J. Pathol. 132:123-146.
Morson, B. C., and J. M. Dawson. 1979. Gastrointestinal pathology. 2nd ed. Blackwell, London.
Nagafuchi, A., Y. Shirayoshi, K. Okazaki, K. Yasuda, and M. Takeichi. 1987. Transformation of cell adhesion properties by exogenously introduced E-cadherin cDNA Nature (Lond.). 329:341-343.
Natt, E., R. E. Magenis, J. Zimmer, A. Mansouri, and G. Scherer. 1989. Regional assignment of the human loci for uvomorulin (UVO) and chymotrypsinogen B (CTRB) with the help of two overlapping deletions on the long arm of chromosome 16. Cyto genet. Cell Genet. 50:145-148.
Nigro, J. M., S. J. Baker, A. C. Preisnijger, J. M. Jessup, R. Hostetter, K. Cleary, S. H. Biggers, N. Davidson, S. Baylin, P. Deville, T. Glover, F. S. Collins, A. Weston, R. Modali, C. C. Harris, and B. Vogelstein. 1989. Mutations in the p53 gene occur in diverse human tumor types. Nat Genet. 3:705-708.
Nomori, H., P. Wallace, J. Johnson, E. M. Earley, S. O'Brien, S. Ferrone, M. A. Pellegrino, J. Milstein, C. Neody, N. Browne, and J. Petricciani. 1979. Characterization of WiDr: a human colon carcinoma cell line. In Vitro (Rockville). 15:401-408.
Nose, A., A. Nagafuchi, and M. Takeichi. 1988. Expressed recombinant cadherins mediate cell sorting in model systems. Cell. 54:993-1001.
Osbourn, M., and K. Weber. 1983. Tumor diagnosis by intermediate filament typing: a novel tool for surgical pathology. Lab. Invest. 48:372-394.
Takahashi, T., M. M. Nan, I. Chiba, M. J. Birrer, R. K. Rosenberg, M. Vinocour, M. Levitt, H. Pass, A. F. Gazdar, and J. Minna. 1989. p53: a frequent target for genetic abnormalities in lung cancer. Science (Wash. DC). 246:491-494.
Taketani, M. 1983. The cadherins: cell-cell adhesion molecules controlling animal morphogenesis. Development (Camb). 102:639-655.
Tsai, Y. C., P. W. Nichols, A. L. Hii, Z. Williams, D. G. Skinner, and P. Jones. 1990. Allelic losses of chromosomes 9, 11, and 17 in human bladder cancer. Cancer Res. 50:64-47.
Tsucha, H., W. Zhang, Y. Shimosato, Y. Yokota, M. Terada, T. Sugimura, T. Miyamura, and S. Hiroshashi. 1990. Allele loss on chromosome 16 associated with progression of human hepatocellular carcinoma. Proc. Natl. Acad. Sci. USA. 87:6791-6794.
Vestweber, D., and R. Kemler. 1984. Rabbit antiserum against a purified surface glycoprotein decompacts mouse preimplantation embryos and reacts with specific adult tissues. Exp. Cell Res. 152:169-178.
Volk, T., and B. Geiger. 1986. A-CAM: a 135-kD receptor of intercellular adhesion junctions. I. Immunoelectron microscopic localization and biochemical studies. J. Cell Biol. 103:1441-1450.
Weidner, K. M., J. Behrens, J. Vandenberkhove, and W. Birchmeier. 1990. Scatter factor: molecular characteristics and effect on the invasion of epithelial cells. J. Cell Biol. 111:2097-2108.
Weinstein, R. S., F. B. Merk, and J. Alroy. 1976. The structure and function of intercellular junctions in cancer. Adv. Cancer Res. 23:23-89.
Yuri, H., A. C. K. Azurina, and J. R. Ruskin. 1977. Human pancreatic carcinoma (MIA PaCa-2) in continuous culture: sensitivity to asparaginase. Proc. Natl. Acad. Sci. USA. 74:2940-2944.
Zbar, B., H. Braith, C. Talmadge, and M. Linehan. 1987. Loss of alleles of loci at the short arm of chromosome 3 in renal carcinoma. Nature (Lond.). 327:721-725.