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Expression of E-cadherin in embryogenetic ingression and cancer invasion

MARC MAREEL[1]*, MARC BRACKE[1], FRANS VAN ROY[2] and LUCIEN VAKAET[1]

[1]*Laboratory of Experimental Cancerology, Department of Radiotherapy and Nuclear Medicine, University Hospital and* [2]*Laboratory of Molecular Biology, University of Ghent, Ghent, Belgium*

ABSTRACT Homophilic interactions of E-cadherin serve the organization of embryonic and adult epithelia and counteract cancer invasion. The role of E-cadherin as an invasion-suppressor molecule has been demonstrated cancer. Regulation of embryonic ingression and cancer invasion via E-cadherin occurs at transcriptional, translational and post-translation levels.

KEY WORDS: *uvomorulin, ingression, invasion, metastasis, human cancer*

Introduction

Cancer development from epithelial cells may proceed along the following scenario: genetic alterations result in loss of growth control leading to the formation of a tumor. This tumor is benign, meaning that the cells stay together. Other genetic alterations result in loss of cell-cell adhesion leading to detachment of cells. Such a tumor is malignant, meaning that it does invade and that it may metastasize (Mareel *et al.*, 1990).

During initial cleavage of the mouse egg, the blastomeres move freely inside the zona pellucida, as is shown by *in vivo* time lapse videomicrography (Fink, 1991). At a given time, compaction occurs by the simultaneous clinging together of the blastomeres. They form an epithelium that builds up the wall of the blastocyst. As described by Monod's group in Paris, this compaction is accompanied by the appearance of a Cell Adhesion Molecule (CAM) on the surface of the cells. The term uvomorulin was coined for the molecule, from *uva* (a bunch of grapes) and *morula* (mulberry). More importantly, antibodies against uvomorulin inhibited the compaction (Hyafil *et al.*, 1980; Peyriéras *et al.*, 1983). This was a promising step towards the biochemical explanation of selective cohesion of cells during embryonic development as demonstrated in the seminal paper by Townes and Holtfreter (1955).

The major difference between embryogenesis and cancer development is that the scenario of the former follows a strict and predictable time schedule, whereas the sequences in the scenario of the latter are unpredictable (Mareel *et al.*, 1991a, 1992; Mareel *et al.*, in press). Among human tumors, colon cancer is about the only one that is suitable for a sequential analysis of the molecular genetics underlying the transition between normal and benign, and between benign and malignant conditions (Fearon and Vogelstein, 1990).

Recently, the compaction-related cell-cell adhesion molecule uvomorulin has been implicated also in cancer development (Behrens *et al.*, 1992; Van Roy and Mareel, 1992). The present review discusses the role of uvomorulin, called here E-cadherin, in embryonic ingression and cancer invasion with emphasis on the transcriptional and post-transcriptional modulation of its expression.

E-cadherin and cell-cell adhesion

The adhesion molecules that have been most intensively studied with respect to epithelial cell-cell adhesion are encoded by genes of the cadherin superfamily (Edelman and Crossin, 1991; Takeichi, 1991; Kemler, 1992). One of these cell-cell adhesion molecules, E-cadherin (identical to or homologous with uvomorulin, L-CAM, Arc-1 and cell CAM 120/180) is expressed in most embryonic and adult epithelia. Moreover, it might serve as an organizer (master molecule) of adherens junctions leading in a cascade of events to epithelial organogenesis (Gumbiner *et al.*, 1988; Magee and Buxton, 1991; Rouiller *et al.*, 1991; Shore and Nelson, 1991).

The locus for human E-cadherin has been mapped to a subregion within band 16q22.1 (Natt *et al.*, 1989). E-cadherin is synthesized from a 4.5-kb mRNA as a 135-kDa precursor polypeptide which is rapidly (2 h) and efficiently (100%) processed to the mature 120-kD form before delivery to the cell surface (Shore and Nelson, 1991). The turnover of E-cadherin at the cell surface has a half life of about 5 h. The mature E-cadherin is an integral membrane glycoprotein with a single membrane spanning domain and an extracellular domain that is implicated in homophilic binding by an as yet unidentified mechanism (Fig. 1). The cytoplasmic domain is noncovalently linked to catenins. Catenins are believed to be parts

Abbreviations used in this paper: CAM, cell adhesion molecule; CAT, chloramphentyl transferase; LOH, loss of heterozygosity; SF/HGF, scatter factor/hepatocyte growth factor.

***Address for reprints**: Laboratory of Experimental Cancerology, Department of Radiotherapy and Nuclear Medicine, University Hospital, De Pintelaan 185, B-9000 Ghent, Belgium. FAX: 32-91-40.49.91.

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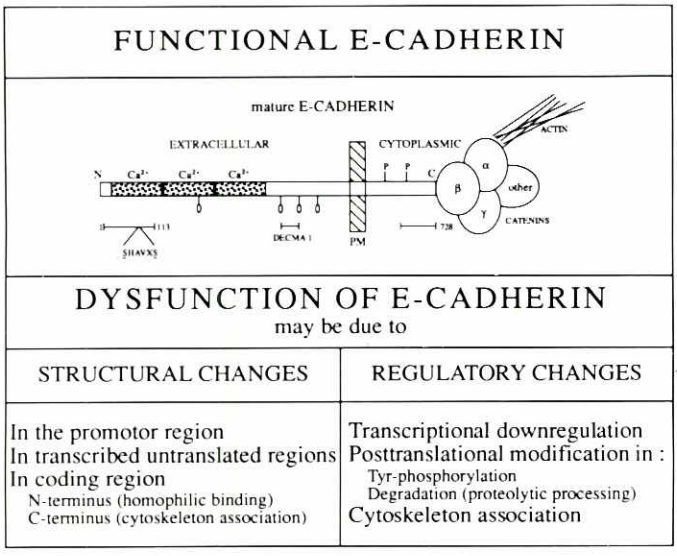


Fig. 1. The molecular domain structure of the 120-kDa functional mature mouse E-cadherin (very high homology in functional domains with human E-cadherin) contains 728 amino acid residues. *N, amino-terminus; C, carboxy-terminus; P, phosphorylation sites on Ser and Thr residues; Ca^{2+}, putative calcium-binding domains; flags are potential glycosylation sites; PM, plasma membrane. The amino-terminal fragments (residues 1 to 113 and, in particular, the underlined amino acid residues) are essential for intercellular binding and determine binding specificity. The DECMA-1 antibody epitope is located centrally near a cystein-rich domain. A highly conserved C-terminal domain essential for stable cell-cell adhesion is underscored. α, β, γ and other indicate catenins. Modified after McCrea and Gumbiner (1991), Ozawa et al. (1990a,b) and Takeichi (1991). The lower part of the figure summarizes possible causes of dysfunction of E-cadherin.*

of a multicomponent submembranous network which connects E-cadherin to other integral membrane proteins and to the cytoskeleton (Nelson *et al.*, 1990; Ozawa and Kemler, 1992). The structure and subcellular localization of E-cadherin is compatible with a role not only in cell-cell adhesion but also in signalling.

To explore the function of E-cadherin various assays have been used. *In vitro*, the following phenotypes, all expressed in presence of Ca^{2+}, point to functional E-cadherin: fast (30 min) cellular aggregation (Takeichi, 1977; Kadmon *et al.*, 1990); compaction, i.e. formation of compact aggregates as compared to loose clusters after overnight incubation (Matsuyoshi *et al.*, 1992); epithelial organization on solid tissue culture substrate (Shore and Nelson, 1991); epithelial organization around fragments of embryonic chick heart in organ culture (Behrens *et al.*, 1989; Vleminckx *et al.*, 1991; Mareel *et al.*, 1992); clustering on top of collagen gels (Vakaet *et al.*, 1991); low dispersion index, i.e. tight colony formation (Matsuyoshi *et al.*, 1992) and formation of glandular structures (Pignatelli *et al.*, 1992) inside collagen gels.

In vivo, formation of epithelial structures is a good indication for expression of functional E-cadherin (Mareel *et al.*, 1991b, 1992; Vleminckx *et al.*, 1991).

Phenotypes expressed in the different assays may reveal different aspects of E-cadherin function. For instance, 3Y1 rat fibroblastic cells formed aggregates in a short term assay as well as compaction after overnight incubation, whereas their *src* -transformed derivatives SR3Y1 scored positive in the fast aggregation assay but not in the compaction assay (Matsuyoshi *et al.*, 1992). MCF-7/6 human breast cancer cells produced epithelial sheets on solid substrate but failed to aggregate in the short term assay, in contrast to their counterpart MCF-7/AZ cells which scored positive in both assays (our unpublished results). It is our opinion that each assay has to be considered as a different micro-ecosystem, the elements of which may or may not influence the expression of the E-cadherin-dependent phenotypes (Mareel *et al.*, 1992).

The E-cadherin-dependence of the expression of the phenotypes in the above-mentioned assays *in vitro* has been demonstrated through inhibition with E-cadherin-specific antibodies (Behrens *et al.*, 1989; Vleminckx *et al.*, 1991; Pignatelli *et al.*, 1992). It should be recalled here that it was antibody-mediated prevention of compaction of preimplantation mouse embryos that led to the first detection of E-cadherin (Hyafil *et al.*, 1980).

In the two step scenario of cancer development, we have considered growth and invasion as unrelated (Mareel *et al.*, 1990). Growth would depend upon inactivation of tumor suppressor genes, and invasion upon loss of cell-cell adhesion molecules among other invasion-promoting events. There are, however, indications that the situation is less simple in as much as cell-cell adhesion molecules may be also implicated in growth, although direct evidence for this implication is missing. Some tumor suppressor gene products were found to possess similarity with cell-cell adhesion molecules. The *Drosophila fat* gene, one of the seven known *Drosophila* tumor suppressor genes, belongs to the cadherin gene superfamily; it encodes a very large transmembrane protein of more than 5, 000 amino acids with 34 tandem cadherin domains, 4 EGF-like repeats, a transmembrane domain and a cadherin-unrelated novel cytoplasmic domain (Mahoney *et al.*, 1991). Recessive mutations in the *fat* locus cause loss of single layered epithelial structure (cell-cell adhesion) and tumor formation (growth) in the larval imaginal discs. The human DCC (deleted in colon cancer) gene has been implicated in growth control of the colon mucosa (Fearon and Vogelstein, 1990; Tanaka *et al.*, 1991). A recent report does, however, implicate the inactivation of the DCC gene in the progression from noninvasive to invasive colon carcinoma on the basis of loss of heterozygosity on chromosome 18q including the DCC locus (Kikuchi-Yanoshita, 1992). Neither for the *fat* gene nor for the *DCC* gene products has it been demonstrated that they actually function as cell-cell adhesion molecules. Since the cytoplasmic domain of the *fat* gene product is different from that of the cadherins, and since an intact cadherin cytoplasmic domain is needed for cell-cell adhesion, one may presume different mechanisms for both types of molecules, at least with regard to adhesion. A relationship between adhesion and growth was also suggested by the experiments of Navarro *et al.* (1991) and of Vleminckx *et al.* (1991); tumors produced by injection of E-cadherin-positive cells did grow more slowly than tumors from E-cadherin-negative cells.

Whether alteration of growth results from direct signalling through cadherin or is an indirect consequence of altered tissue organization remains to be examined.

Patterns of E-cadherin expression

During avian gastrulation, cells of the upper layer lose their coherence and ingress through the primitive streak. Studying the

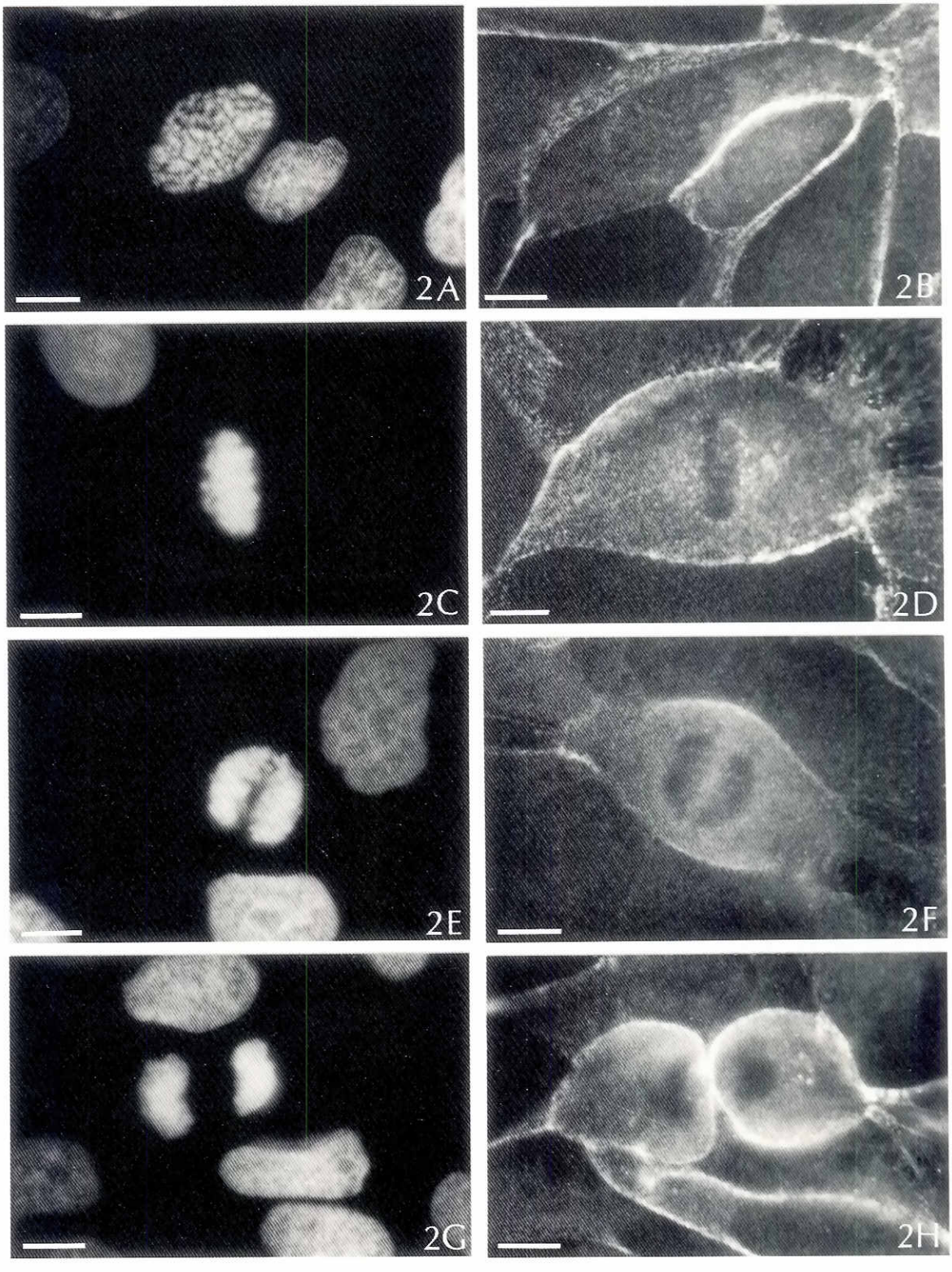


Fig. 2. Staining of MDCK canine kidney cells in culture on glass with DAPI (panels A, C, E and G) and with antibody DECMA-1 followed by FITC-conjugated sheep anti-mouse IgG (panels B, D, F and H). *The figure shows consecutive stages of mitosis: prophase* **(A and B)**; *metaphase* **(C and D)**; *anaphase* **(E and F)**; *telophase* **(G and H)**. *The E-cadherin immunosignal of the mitotic cells is in focus slightly above the immunosignal of the interphase cells. Scale bar= 10 μm.*

chicken blastoderm at stage 9 (Vakaet, 1970), Edelman *et al.* (1983) showed immunohistochemically that L-CAM was present between the cells of the upper layer in the groove of the primitive streak. Fig. 1c in the latter paper clearly shows that L-CAM is present all around the cells in mitosis. This is what we found also for MDCK cells in culture on glass (Fig. 2). L-CAM was still present on recently ingressed interphase middle layer cells. Later, the loss of cell-cell adhesion leading to detachment of the middle layer cells was accompanied by loss of the expression of L-CAM. During organogenesis, in some tissues cell-cell adhesion may be reinstated. One example is the formation of nephric tubules out of mesenchyme. Vestweber *et al.* (1985) looked for the presence of uvomorulin in metanephrogenic tissue of rabbits during experimental induction of tubules. They found that 12 h after induction, uvomorulin was present in the formed tubules but not before. The authors did not succeed in preventing formation of tubules by anti-uvomorulin antibodies, although they demonstrated that the antibodies had penetrated into the tissue and were bound to the uvomorulin. They concluded that other cell surface molecules may be sufficient for this morphogenetic process.

Since the paper on the expression of E-cadherin in a variety of human tumors and normal tissues published by Eidelman *et al.* (1989), a number of reports have appeared including cancers originating from the following tissues: skin (Czech *et al.*, 1990); breast (Shiozaki *et al.*, 1991); upper respiratory and alimentary tract, head and neck, (Schipper *et al.*, 1991); esophagus (Shiozaki *et al.*, 1991); gastric mucosa (Shimoyama and Hirohashi, 1991; Shiozaki *et al.*, 1991); lung (Shimoyama *et al.*, 1989); arachnoid villi (Tohma *et al.*, 1992); colon (Van Aken *et al.*, 1991; Dorudi *et al.*, 1992); prostate (Bussemakers, 1992). In these tissues, immunostaining with an E-cadherin-specific antibody shows homogeneous positivity in the epithelia of origin and in benign tumors (e.g. polyps) and heterogeneity in malignant tumors with quantitative and qualitative changes of the immunosignal (Fig. 3). Alterations of E-cadherin are most obvious in the less well differentiated tumors which are also the more aggressive ones (Mareel *et al.*, 1991a; Gabbert *et al.*, 1992). Taken together these data show alterations in E-cadherin expression in malignant, i.e. invasive, cancers in agreement with the idea that E-cadherin is an invasion-suppressor molecule (Behrens *et al.*, 1992; Van Roy and Mareel, 1992). We should, however, consider that immunostaining is instructive about the presence of the antigen but does not explore the function of the molecule. Furthermore, these static observations do not tell us wether or not expression of E-cadherin is the key event in epithelial organization and whether or not down-regulation of E-cadherin is responsible for ingression and invasion.

Experimental evidence in favor of a master role for E-cadherin

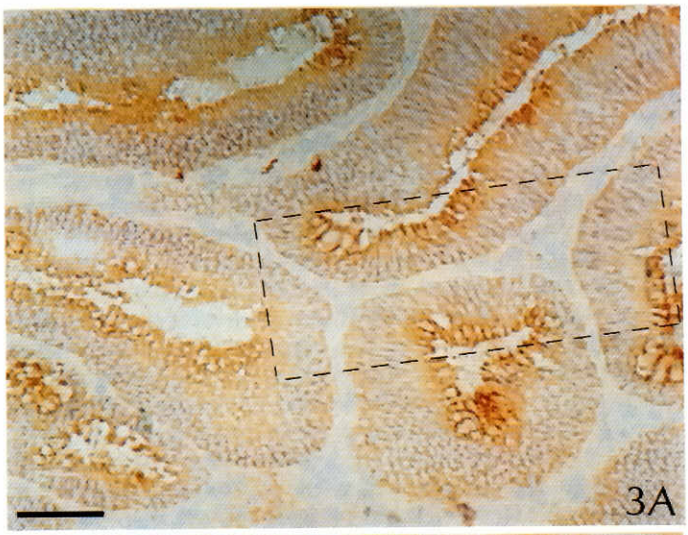
For cancer development, 4 types of experimental manipulations *in vitro* have provided arguments in favor of an invasion-suppressor role of E-cadherin in various epithelium-derived cell types (Behrens *et al.*, 1989, 1992; Chen and Öbrink, 1991; Frixen *et al.*, 1991; Vleminckx *et al.*, 1991; Mareel *et al.*, 1992b; Van Roy and Mareel, 1992). First, a negative correlation was found between the expression of E-cadherin and invasion *in vitro* for several families of animal cell lines and for a variety of human cancer cell lines. Second, in E-cadherin-negative cancer cell types, invasion could be abrogated by transfection and expression of exogenous E-cadherin cDNA. Third, reduction of E-cadherin mRNA levels after transfection with a plasmid expressing E-cadherin-specific antisense RNA induced the invasive phenotype in E-cadherin-positive and invasion-negative cells. Fourth, in cells expressing the endogenous or the exogenous E-cadherin at high and homogeneous levels, invasion could be induced by addition of some antibodies inactivating functional E-cadherin. Note that not all antibodies are functionally inactivating despite their specific binding and that the antigen cannot always be reached by the antibody. In cell lines derived from a normal murine mammary gland, E-cadherin-negative cells only became invasive after introduction of a powerful oncogene like *ras* (Vleminckx *et al.*, 1991). The invasive behavior of these cells was suppressed by transfection with E-cadherin cDNA, whereas their levels of *ras* expression remained the same, indicating that E-cadherin may counterbalance the invasion promoter activity of oncogenes. How such oncogenes induce the expression of the invasive phenotype and at what level this is counterbalanced by E-cadherin is an open question (Mareel and Van Roy, 1986; Van Roy and Mareel, 1992).

Levels of E-cadherin regulation

Cadherins are plasma membrane receptors, sensitive to regulation at various levels.

In tumors no mutations have been found for cadherins, so far, although mutant E-cadherins have been expressed in cultured cells (Ozawa *et al.*, 1990). Loss of heterozygosity (LOH) at specific chromosomal locations is accepted as an indication of a tumor suppressor gene at these locations. In prostate cancer, LOH at chromosome 16q was a frequent (30%) event (Carter *et al.*, 1990). LOH at 16q was found also in 3 out of 25 tumors from the neurectoderm (Thomas and Raffel, 1991) and in breast cancer, allele loss on 16q correlated positively with lymph node metastases (Sato *et al.*, 1990). In hepatocellular carcinomas, a tumor suppressor gene has been localized on chromosome 16q22.1 to 16q23.2, i.e. the region where the E-cadherin gene is located (Tsuda *et al.*, 1990). The late loss of this allele during hepatocarcinogenesis is consistent with an invasion suppressor role of E-cadherin in these tumors. DNA sequences sensitive to tissue-specific regulatory elements have been identified in the E-cadherin promoter (Behrens *et al.*, 1992); in a CAT (Chloramphenicol Acetyl Transferase) reporter assay, these sequences were found to be highly active in differentiated breast carcinoma cells but not in undifferentiated carcinoma cells. One major problem with genomic alterations is that they are seldom unique but mostly associated with multiple others on the

Figs. 3 to 6. E-cadherin immunohistochemistry of different human colon tumors, namely adenoma (3), well-differentiated (4); moderately differentiated (5) and poorly differentiated (6) carcinoma. *Panels B show details of boxed-in areas of panels A. Frozen sections were treated with a mouse monoclonal antibody against human E-cadherin (MLCA; Euro-Diagnostics B.V., Apeldoorn, The Netherlands) followed by a modified PAP technique. Scale bars= 50 μm.*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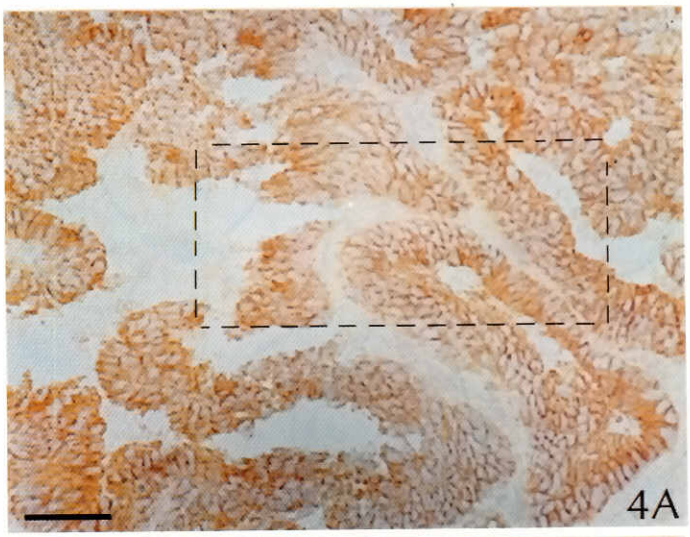
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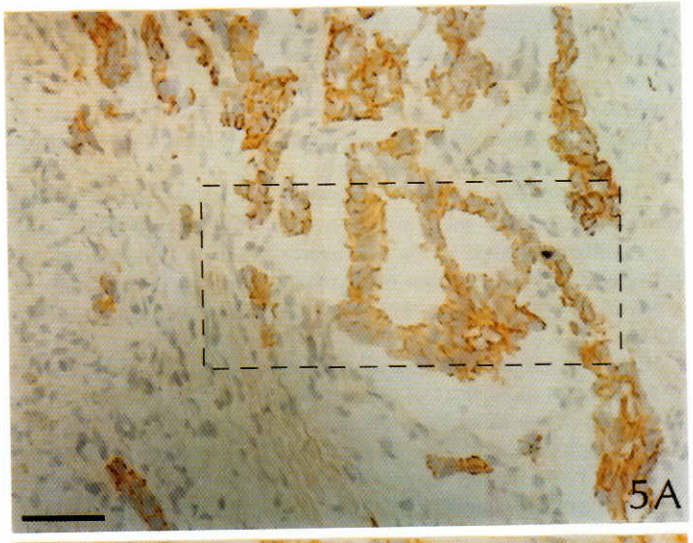
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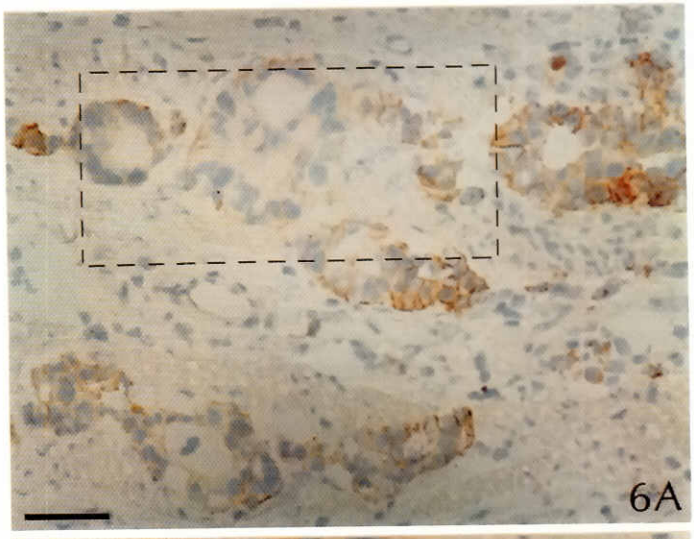
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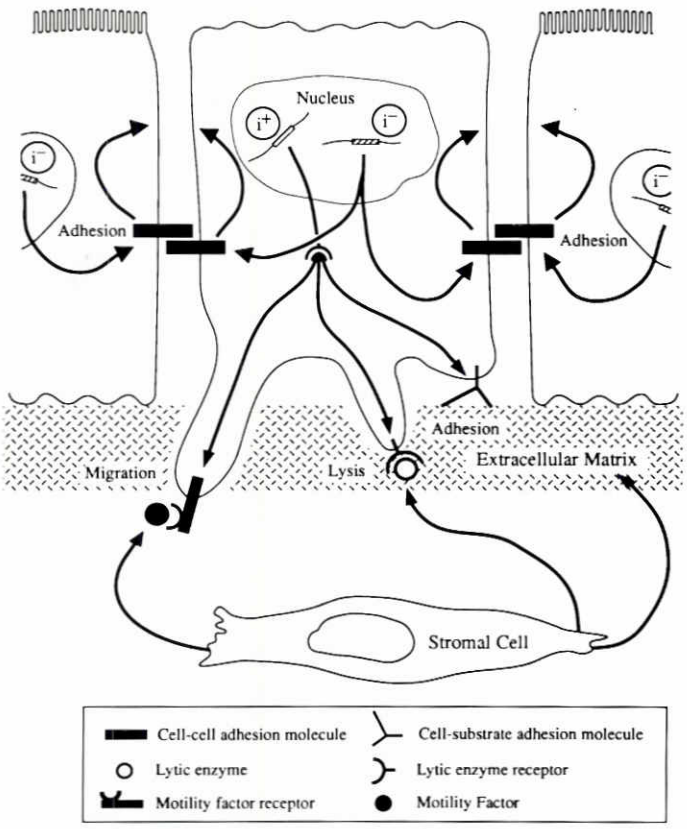

Fig. 7. Schematic representation of a micro-ecosystem of invasion/ingression; *i⁺= invasion (or ingression)-promoter gene, encoding molecules that govern the expression of the invasive phenotype; i⁻= invasion (or ingression)-suppressor gene, encoding molecules that govern the organization of coherent epithelial structures. Adapted from Mareel et al. (1991a).*

same or on other chromosomes. This makes it difficult to establish causal relationships between chromosomal changes and tumor phenotypes, and it has even been proposed that the accumulation of allelic deletions is more important than the order in which they occur (Fearon and Vogelstein, 1990). Developmentally regulated expression of cadherin by trans-acting factors can be analyzed with the dominant mutants that have been derived from compaction-competent embryonal carcinoma cell lines but that lack E-cadherin expression and that have lost the compaction-competence (Weng and Littlefield, 1991).

With experimental tumors, down-regulation of both endogenous and transfected E-cadherin expression has been demonstrated after injection of homogeneously E-cadherin-positive cell populations into host animals (Mareel *et al.*, 1991b; Vleminckx *et al.*, 1991; Gao *et al.*, 1992). In one of these experiments down-regulation was shown to occur at the transcriptional level (Gao *et al.*, 1992). Mesenchymal cells from a fetal mouse are at the origin of the MO_4 cell line. The latter cells did not express E-cadherin, probably because of permanent down-regulation during embryonic development. Transfection of MO_4 cells with E-cadherin cDNA resulted in the isolation of MO_4 cells that did express exogenous E-cadherin at their surface. The molecule seemed to be functional in as much as the transfectants, in contrast with the parental cells, showed Ca^{2+}-dependent fast aggregation and did not invade into collagen gel. When E-cadherin-positive MO_4 cells were injected into syngeneic mice, they produced invasive and metastatic tumors in which no E-cadherin-positive cells could be detected by immunohistochemistry. By Northern blotting it was shown that the levels of E-cadherin mRNA were at least 10 times lower in the tumors than in the cells that were injected. When these tumors were brought into culture, cells started to re-express E-cadherin readily. These observations indicate down-regulation of E-cadherin at the transcriptional level under the influence of host factors. It is puzzling that this down-regulation occurs despite the fact that the transfected E-cadherin is under the control of a constitutive ß-actin promoter (Nagafuchi *et al.*, 1987). Temporary down-regulation of E-cadherin in invasive rat prostate cancers with re-expression in some parts of the metastases formed occurred also at the transcriptional level as evidenced by Northern blot analysis (Bussemakers *et al.*, 1992). However, in human prostate cancer, no correlation was found between E-cadherin mRNA levels and the E-cadherin immunosignal (Bussemakers, 1992). Using *in situ* hybridization, a technique that takes into account heterogeneity within a single tumor, Schipper *et al.* (1991) and Dorudi *et al.* (1992) did find a correlation between low E-cadherin immunosignal and reduced levels of mRNA in human head and neck tumors and in human colon cancers, respectively.

Post-translational modulation of E-cadherin, with or without alteration of the immunosignal, is an alternative to transcriptional regulation and can occur at various levels (see Fig. 1). Since E-cadherin is a glycoprotein of the complex type, glycosylation may be a way of fine tuning, too. Experiments with tunicamycin-treated MDCK cells have led to the conclusion that core or complex carbohydrates are not required for processing and transport to the surface of E-cadherin (Shore and Nelson, 1991). The role of the carbohydrate residues in the adhesion or the signalling function of E-cadherin remains to be explored.

Ca^{2+} is needed for the stabilization of cadherins and for their correct assembly into adherens junction complexes. Since extracellular calcium levels are known to vary within tissues, this provides another possible mechanism of regulation of cadherin, e.g. during epithelial-mesenchymal transition (Magee and Buxton, 1991).

Catenins are directly associated with the cytoplasmic domains of E-cadherin as well as of other members of the cadherin family. Members of the catenin family are: α-catenin (102 kDa), showing homology to vinculin (Herrenknecht *et al.*, 1991); ß-catenin (88 kDa), which is homologous to the *Drosophila armadillo* gene product (McCrea and Gumbiner, 1991) and distinct but closely related to plakoglobin (Butz *et al.*, 1992); γ-catenin (80 kDa), the molecular structure of which has not yet been characterized. Lack of binding to catenins results in loss of the cell-cell adhesion function of E-cadherin mutants, despite their expression at the cell surface (Nagafuchi and Takeichi, 1989; Ozawa *et al.*, 1990b). αN-catenin is a recently identified subtype of α-catenin (now termed αE-catenin) that is expressed mainly in the nervous system where it is associated with N-cadherin. Recent work with *Xenopus* embryos indicated that the cytoplasmic domains of different cadherins may compete for binding to catenins (Kintner, 1992). During early cleavage in *Xenopus*, the surface epithelium expresses P-cadherin; later, the

animal pole ectoderm produces epidermal epithelium and neuroepithelium expressing E-cadherin and N-cadherin respectively (Hatta and Takeichi, 1986; Choi and Gumbiner, 1989; Choi *et al.*, 1990; Angres *et al.*, 1991; Ginsberg *et al.*, 1991; Levi *et al.*, 1991). Injection of large amounts of N-cadherin RNA into the animal pole caused loss of cell-cell adhesion suggesting that N-cadherin disturbed the function of E-cadherin (Detrick *et al.*, 1990). The same result was obtained with a mutant form of N-cadherin coined N-CadΔE that contained a signal peptide, the transmembrane and the intracellular domain but completely lacked the extracellular part of the molecule. Inhibition of cell-cell adhesion in *Xenopus* ectoderm both in the embryo and *in vitro* was explained through competition between E-cadherin and N-CadΔE for binding to catenins. Such competition may regulate cell-cell adhesion in tissues that transiently express different types of cadherin during morphogenesis. Lung carcinoma PC9 cells, expressing E-cadherin at their surface and ß-catenin in their cytoplasm but neither α-catenin nor αN-catenin, grew as isolated cells (Hirano *et al.*, 1992). When these cells were transfected with αN-cadherin cDNA they formed aggregates showing epithelial and sometimes also cystic organization. The authors concluded that αN-catenin was crucial not only for E-cadherin-mediated cell-cell adhesion but also for the organization of multicellular structures. How the E-cadherin/catenin complex fulfils this task is not understood.

There are observations that open new and intriguing ways of regulating E-cadherin function but the molecular mechanisms remain to be elucidated.

We demonstrated E-cadherin at the surface of human MCF-7/6 mammary carcinoma cells by immunocytochemistry with different monoclonal antibodies. Nevertheless, MCF-7/6 cells failed to aggregate in the presence of Ca^{2+}, and they were invasive into embryonic chick heart fragments in organ culture (our unpublished observations). This indicates that E-cadherin was not functional in MCF-7 cells with regard to cell-cell aggregation and invasion. Treatment of MCF-7/6 cells with 0.5 μg/ml insulin-like growth factor I (IGF-I) led to Ca^{2+}-dependent aggregation and to inhibition of invasion *in vitro*. The effect of IGF-I on cellular aggregation did not necessitate *de novo* protein synthesis, as evidenced by cycloheximide treatment. Monoclonal antibodies that bound to either the IGF-I receptor or E-cadherin abrogated the effect of IGF-I on Ca^{2+}-dependent fast aggregation. These results indicate that the function of E-cadherin can be regulated by IGF-I at least in MCF-7/6 cells by an, as yet, unknown mechanism.

The motogenic mitogen, called scatter factor/hepatocyte growth factor (SF/HGF), induced loss of epithelial organization of several types of cells on solid substrate and invasion into collagen type 1 gels; it did not, however, affect the steady state level, stability or overall phosphorylation of E-cadherin (Weidner *et al.*, 1990). Possible explanations are: the signal cascade triggered by SF/HGF bypasses the E-cadherin-mediated cell adhesion system, or SF/HGF causes as yet unidentified alterations in E-cadherin or in its associated proteins.

Conclusions

Circumstantial and experimental evidence indicates that the cell-cell adhesion molecule E-cadherin (also called uvomorulin) counteracts embryonic ingression and cancer invasion. In the micro-ecosystem, in which invasion and ingression are governed by the balance between the activation of promoter and suppressor genes, E-cadherin is the first well documented suppressor gene product (Fig. 4). Such a balance may act in a direct way. In such cases, the promoter gene product functionally inactivates the suppressor gene product or *vice versa*. The balance may also act in a more indirect and general way. In such cases, changes that are unrelated to the above-mentioned promoter or suppressor genes may, nevertheless, interfere with the proper function of the gene products. Our continued interest in the invasion/ingression-suppressor role of E-cadherin is motivated by E-cadherin's domain organization permitting regulation at multiple post-translational levels besides the transcriptional level, and by E-cadherin's activity as a master molecule regulating the formation of epithelial junctional complexes.

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