Molecular Biology Reports **19**: 45–67, 1994.

Invasion promoter versus invasion suppressor molecules: the paradigm of E-cadherin

Marc Mareel[1], Marc Bracke[1] & Frans Van Roy[2]
Department of Radiotherapy, Nuclear Medicine and Experimental Cancerology, University Hospital, De Pintelaan 185, B-9000 Ghent, Belgium
[2]*Laboratory of Molecular Cell Biology, Department of Molecular Genetics, University of Ghent, K.L. Ledeganckstraat 35, B–9000 Ghent, Belgium*

Received and accepted 8 February 1993

Key words: invasion, metastasis, cell adhesion, E-cadherin, L-CAM, uvomorulin, IGF-I

Introduction

Metastasis usually makes cancer incurable and is, therefore, a major determinant of the malignancy of this disease [1]. Benign tumors remain constraint to the proper domain of their tissue of origin. Local invasion, however, marks the onset of malignancy in human cancer. Malignant solid tumors form a spectrum from close-to-benign to extremely aggressive. Basocellular epitheliomas are invasive but not metastatic and they are cured in close to 100% of all cases. Primary tumors of the brain are invasive but rarely metastatic. Nevertheless, some of them are marked by a 2-year survival rate of not more than 10%. Head and neck cancers frequently kill through local invasion with involvement of the lymph nodes but without metastasis to distant organs. Improvement of local tumor control implicating longer survival has led to an increase in the frequency of distant metastases [2]. Melanomas frequently show limited local growth; they are invasive and form metastases in a large variety of organs. For the majority of human cancers, aggressiveness in terms of invasion and metastasis is situated between that of head and neck cancers and that of melanomas. For individual cancers, the TNM staging (T, primary tumor; N, lymph node; M, distant metastasis) is used for prognosis (TNM Atlas). For T staging depth of invasion is a major determinant.

Current concepts describe metastasis as a multistep process of invasion [3]: invasion from the primary cancer mostly through the basement membrane into the surrounding tissues; intravasation into lymph and blood; transport inside the circulation with the sinusoids of lymph nodes and with the capillary beds of lungs or liver as the first obstacles; extravasation at the site of lymph node or visceral metastasis. Metastases are invasive tumors as well and may repeat this multistep process, initiating the metastatic cascade. At each step of the process of metastasis we consider the expression of the invasive (I-plus) or the noninvasive (I-minus) phenotype within the frame of a micro-ecosystem (for example, see Fig. 1). Such micro-ecosystems consist of neoplastic cells and of a variety of host cells and extracellular matrix. Although such micro-ecosystems are initiated by the neoplastic cells, we consider the elements of the host as equally important partners in the expression of the invasive phenotypes [4]. This expression is believed to result from a balance between acti-

vation and inactivation of *i*-minus/invasion suppressor genes and *i*-plus/invasion promoter genes [5, 6]. The present review covers current knowledge about the expression of such genes with special emphasis on one particular suppressor gene product, namely the cell-cell adhesion molecule E-cadherin.

Growth, governed by oncogenes and tumor suppressor genes, is regulated separately from the invasive phenotype [4]. Examples of oncogene products are found mainly in families of growth factors, of growth factor receptors and of growth signal transducers. A famous example of a tumor suppressor gene product is the growth regulatory nuclear protein p53 [7]. Like invasion, growth regulation may fall also under the control of micro-ecosystems. For example, growth of cancer cells at the site of metastasis may be controlled by paracrine organ-derived and sometimes organ-specific growth factors [8, 9]. In analogy with growth-regulating oncogenes and tumor suppressor genes, we have presumed that invasion promoter and invasion suppressor genes do exist encoding invasion-regulating molecules.

Tumor development starts with genetic changes in a cell that will eventually give rise to a neoplastic cell population. This neoplasia manifests itself when it has grown up to a sizable extent. Progression to full malignancy implicating invasion and metastasis is believed to result from multiple genetic changes. It is our hypothesis that progression can also be described in terms of micro-ecosystems in which the host cells participate (Fig. 2). Questions such as 'When are invasion promoter genes activated?' and 'When are invasion suppressor genes inactivated?' may then be phrased as 'When do invasion promoter systems become sensitive to upregulation?' and 'When do invasion suppressor systems become sensitive to downregulation?'. The transition from an '*in situ* carcinoma' to an 'invasive carcinoma' may then be described as the transition from a micro-ecosystem 'not permissive for invasion' toward a micro-ecosystem that is 'permissive for invasion'.

Invasion promoter genes

Invasion promoter or *i*-plus genes encode molecules that have a regulatory function in the expression of the invasive (I-plus) phenotype, i.e., the migration of cancer cells from one tissue domain into another. Such molecules may be implicated in a variety of cellular activities, such as migration, lysis of the extracellular matrix, cell-matrix adhesion and heterotypic cell-cell adhesion. These cellular activities have been recently reviewed [10] and we shall add here a few more examples of how they may be regulated.

Migration

Migration is regulated by the expression of motility factor receptors on the cancer cell and by autocrine (neoplastic cell) or paracrine (host cell) secretion of motility factors. Examples are autocrine motility factor (AMF) with its gp78 receptor, and scatter factor/hepatocyte growth factor (SF/HGF) with its c-*met* receptor. AMF is sensitive to downregulation by retinoic acid at least in some of cells types and this may well explain its invasion and metastasis suppressor activity [11]. SF/HGF is a growth promoter for some cells and may act as a motility promoter for other cells [12]. It would therefore be expected to be a mediator of the I-plus phenotype. Indeed, SF/HGF was found to stimulate invasion of cancer cells in some micro-ecosystems [13], although not in others [14]. Furthermore, in yet another system SF/HGF-mediated motility seems to serve the expression of the I-minus phenotype, i.e., the organisation of normal tissues. Expression of c-*met* was found to be prominent at the microvilli of the glandular lumina in the normal breast. In breast cancer the expression was less pronounced and restricted to the glandular parts of the tumor [15]. One explanation, given by the authors, is that epithelial scattering is needed for lumen formation. Why in such case c-*met* expression is restricted to the microvilli is not clear.

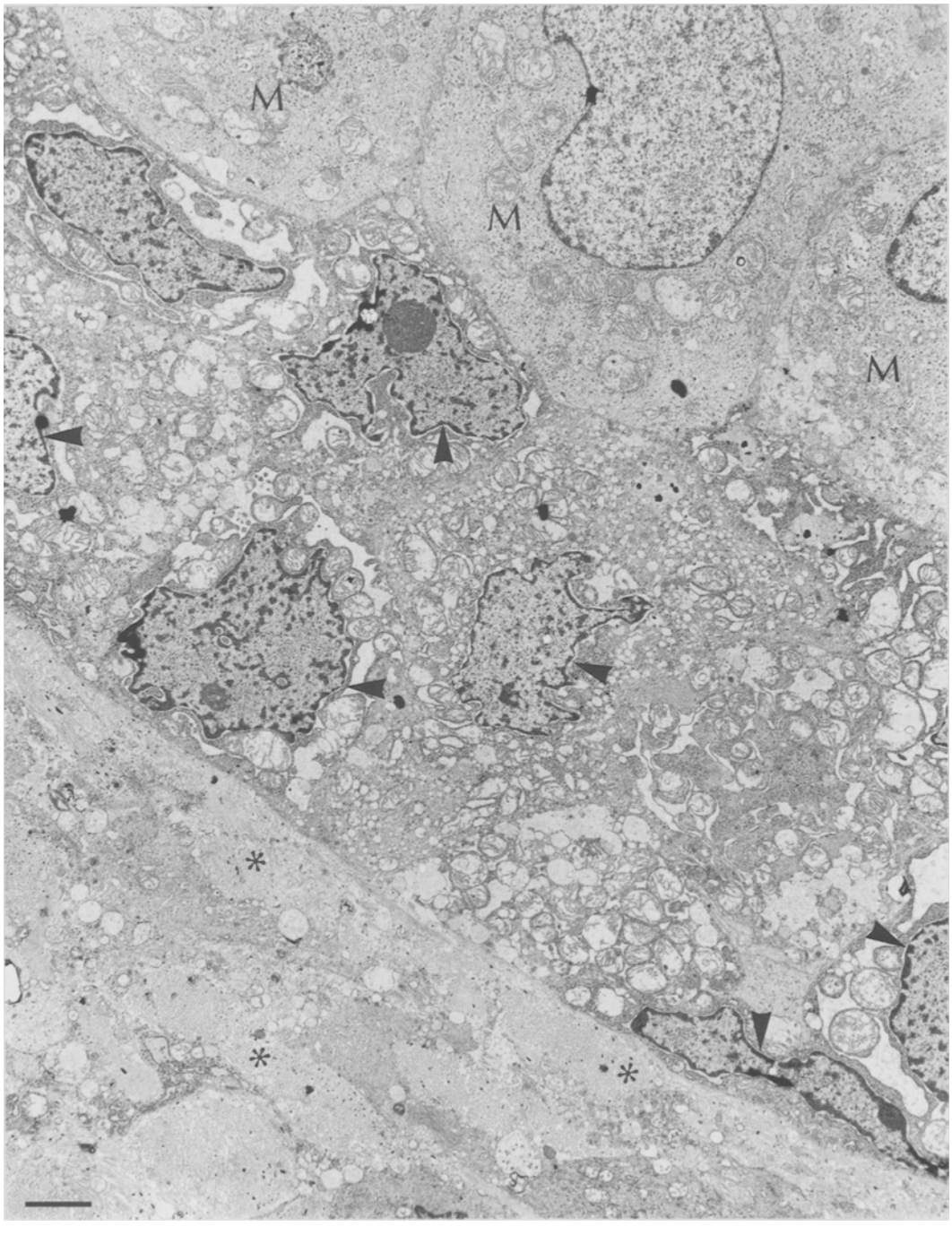


Fig. 1. Transmission electron micrograph from a subcutaneous tumor produced by human MCF-7/AZ cells injected into a nude mouse; elements of the micro-ecosystem shown are: desmoplastic reaction with fibroblasts (arrow heads point at fibroblast nuclei) and extracellular matrix (asterisks); epithelial MCF-7/AZ cells (M). Scale bar = 2 μm.

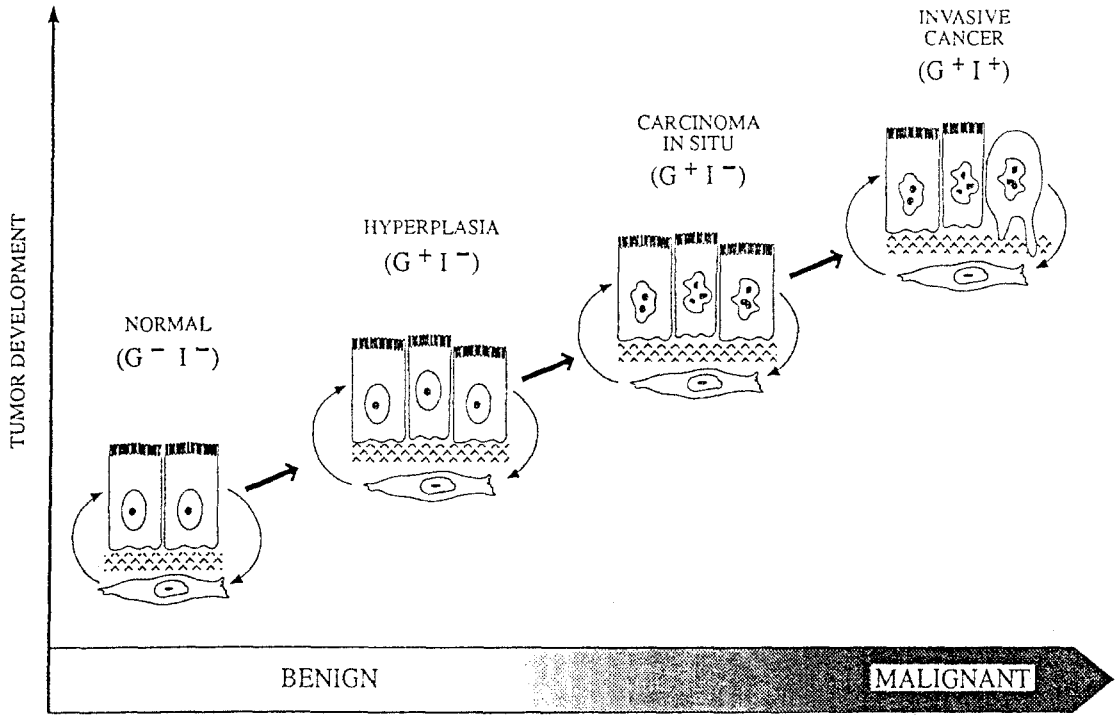


Fig. 2. Schematic representation of cancer development in view of the micro-ecosystem concept. Stages from normal epithelium to invasive cancer are defined in terms of the phenotype expressed by the neoplastic cells and their relationship to the underlying basement membrane. The fusiform cells represent the host cells; they are believed to be influenced by the neoplastic cell population (downward arrows) but they may in turn also influence the development of that neoplastic cell population (upward arrows). Not indicated are autocrine loops that may exist in both the host cell and the neoplastic cell populations.

Breakdown of extracellular matrix

Breakdown of the epithelial or of the endothelial basement membrane is a prerequisite for primary invasion as well as for intravasation and extravasation of cancer cells [16]. Here, the molecular scenario resembles the one for migration in as much as hydrolases secreted by host cells may bind to receptors on the cancer cells and by this way become focally active. Examples are plamin [17] and plasminogen activators (u-PA and t-PA) with their receptors, and matrix metalloproteinases (MMPs) and their binding sites. The activity of these enzymes is regulated not only by focalization but also through the conversion of an inactive into an active form and through specific inhibitors. Amongst the latter, TIMPs are the most notable examples. This scenario does of cause not exclude other mechanisms of action of hydrolases. In human cancers, hydrolases and their competitors have been used as markers of aggressiveness. The following correlates may serve as examples: secretion of the MMP stromelysin–3 by mammary fibroblasts and transition from *in situ* carcinoma toward invasive cancer [18]; enhanced activity of the serine protease u-PA and increased risk for recurrence in breast cancer [19]; levels of 72-kDa type IV collagenase and metastasis in lung cancer [20].

Adhesion to extracellular matrix

Cancer cell-matrix adhesion is regulated by extracellular matrix receptors. Such interaction between cells and extracellular matrix is not limited to adhesion but also implicates signal transduction with regulation of gene expression. Qualitative and quantitative changes of elements of the matrix or of the matrix receptors have been related to invasion [reviewed in ref. 21]. Clinical exam-

ples of such relationships are: increased amounts of $\alpha_6\beta_4$ integrin with aggressive types of squamous cell carcinoma [22] and expression of $\alpha_4\beta_1$ and $\alpha_v\beta_3$ with melanoma progression [23].

Heterotypic cell-cell adhesion

At the site of the primary tumor, heterotypic adhesion between melanoma cells and tumor infiltrated lymphocytes may increase the aggressiveness of human melanoma [24].

Adhesion to the endothelial cell surface is believed to govern extravasation of both normal leukocytes and cancer cells [25]. Hynes and Lander [26] have recently presented an interesting scenario for the adhesion of leukocytes to the vascular endothelium. A leukocyte, expressing at its surface Lewis^x-leukosialin next to integrin receptors, passes along an endothelium. When such an endothelium is activated by inflammatory agents cell-cell adhesion molecules are expressed by the endothelial cells. In consequence, leukocytes are trapped by binding to sialyl Lewis^x, and roll over the endothelium. This slower motion of leukocytes permits further dynamic interaction with the endothelium implicating activation of cell-cell adhesion molecules, that mediate stable adhesion and lead to extravasation. Molecules that are best documented for their role in cell adhesion may also serve as a docking site for other molecules. Cytokines such as macrophage inflammatory protein (MIP–1β) can be sequestered at the surface of the endothelium and by this way trigger events that lead to adherence and eventual extravasation of leukocytes [27]. One candidate for immobilizing MIP–1β at the endothelial cell surface is CD44, variants of which are shown to be involved in metastasis [27, 28].

Invasion suppressor genes

Invasion suppressor or *i*-minus genes are believed to encode molecules that have a master function in the maintenance of normal tissue architecture: i.e., the I-minus phenotype which, obviously, precludes invasion. One of such molecules, though certainly not the only one, is the cell-cell adhesion molecule E-cadherin. We shall restrict ourselves, here, to a discussion of E-cadherin, the choice of which is based on its early and ubiquitous occurence at the epithelial cell-cell adherens junctions.

Structure and function of E-cadherin

Cell-cell adhesion molecules encoded by genes of the cadherin superfamily have been intensively studied with respect to the organisation of embryonic and adult tissues [29–31]. 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 epithelia. Moreover, it might serve as an organizer (master molecule) of adherens junctions leading *via* a cascade of events to epithelial morphogenesis [32–35]. The locus for human E-cadherin has been mapped to a subregion within band 16q22.1 [33]. E-cadherin is synthesized from a 4.5-kb mRNA as a 135-kDa precursor polypeptide which is rapidly (2h) and efficiently (100%) processed to the mature 120-kD form [33]. It is delivered in its mature form to the cell surface, where it 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. 3). The cytoplasmic domain is noncovalently linked to catenins. Catenins are members of protein families involved in cytoplasmic anchorage of cell adhesion molecules. They are believed to be parts of a multicomponent submembranous network which connects E-cadherin to other integral membrane proteins and to the cytoskeleton [36, 37]. Catenins are directly associated with the cytoplasmic domains of members of the cadherin family and coprecipitate with cadherins in immunoprecipitation experiments. The following molecules belong to the catenin family: α-catenin (102 kDa), showing homology to vinculin [38]; β-catenin (88 kDa), which is homologous to the Drosophila *armadillo* (*arm*) gene product [39] and distinct from but closely related to plakoglobin [40]; γ-

catenin (80 kDa), the molecular structure of which has not yet been characterized. The structure and subcellular localisation of E-cadherin is compatible with a role not only in cell-cell adhesion but also in signal transduction.

To explore the function of E-cadherin various assays have been used. A number of phenotypes all expressed *in vitro* in the presence of Ca^{2+} point to functional E-cadherin. Fast (30 minutes) cellular aggregation under Gyrotory shaking as evident from direct observation and from Coulter counting (Fig. 4) probably reveals early steps of cell-cell contact [41, 42]. Inhibition by E-cadherin-neutralizing antibodies demonstrates the necessity for functional E-cadherin but does not exclude that fast aggregation is initiated or completed by other molecules. Shaking under controlled shear force following fast aggregation is used to assess the strenght of E-cadherin-mediated cell-cell adhesion [43]. Compaction, i.e., formation of compact aggregates as compared to loose clusters after overnight culture, reflects a function of E-cadherin during early embryogenesis as discussed below [44]. Segregation of E-cadherin-positive cells from a 1:1 mixture with negative cells may mimic an early step in organogenesis [45]. Epithelial organization on solid tissue culture substrate not only implicates cell-cell but also cell-substrate interactions [33]. With regard to this, it is interesting to notice that the cell-cell connection via E-cadherin and α-catenin leads to the actin cytoskeleton and that this is also the case for the cell-substrate connection via integrins and vinculin [46]. Under the conventional light microscope, various patterns of E-cadherin immunostaining can be observed (Fig. 5). Epithelial organisation of cells, sometimes with formation of a basement membrane, around fragments of embryonic chick heart in organ culture closely resembles the expression of the I-minus phenotype *in vivo* [47–49]. Aggregation on top of a collagen gel without entering into the gel is another index for the I-minus phenotype [50]. When cells are mixed with the collagen before gelation, they form tight colonies or glandular structures inside the gel when E-cadherin-positive, whereas they scatter when E-cadherin-negative [44, 51]. All the abovementioned assays consider exclusively homotypic cell-cell contacts (between cells of the same type) because it has always been firmly believed that E-cadherin served only homotypic interactions. Recently, however, heterotypic binding of solitary cells to monolayer culture has been used to demonstrated E-cadherin-mediated interactions between Langerhans' cells of the skin and keratinocytes [52]. *In vivo* formation of epithelial structures is a good indication for expression of functional E-cadherin [1, 48, 49].

In these experiments, cells were mostly injected as single cell suspensions in which case the formation of cysts, glands or tubules demonstrates the active nature of the expression of the I-minus phenotype. Phenotypes expressed in these different *in vitro* and *in vivo* assays may reveal different aspects of E-cadherin function. For instance, *src*-transformed rat cells scored positive in the fast aggregation assay but negative in the compaction assay [44]. MCF-7/6 human breast cancer cells produced epithelial sheets on solid tissue culture substrate but failed to aggregate in the 30-minute assay, in contrast with their counterpart MCF-7/AZ cells that 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 [53].

The E-cadherin-dependence of the expression of the phenotypes in the abovementioned assays *in vitro* has been demonstrated through inhibition with E-cadherin-specific antibodies [47, 48, 51]. 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 [54]. Such experiments demonstrate that E-cadherin is operative in the expression of the phenotype under consideration and that there is no biological redundancy, but they do not mean that E-cadherin is sufficient.

We have considered above that growth and invasion are activities of cancer cells that are regulated separately. Growth would depend upon inactivation of tumor suppressor genes, and invasion upon loss of *i*-minus gene products such as

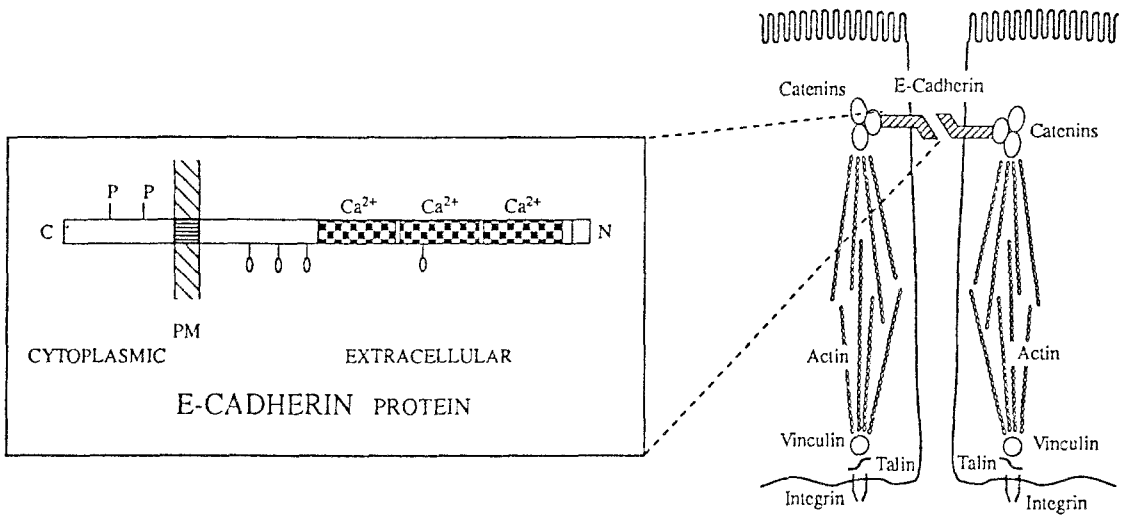
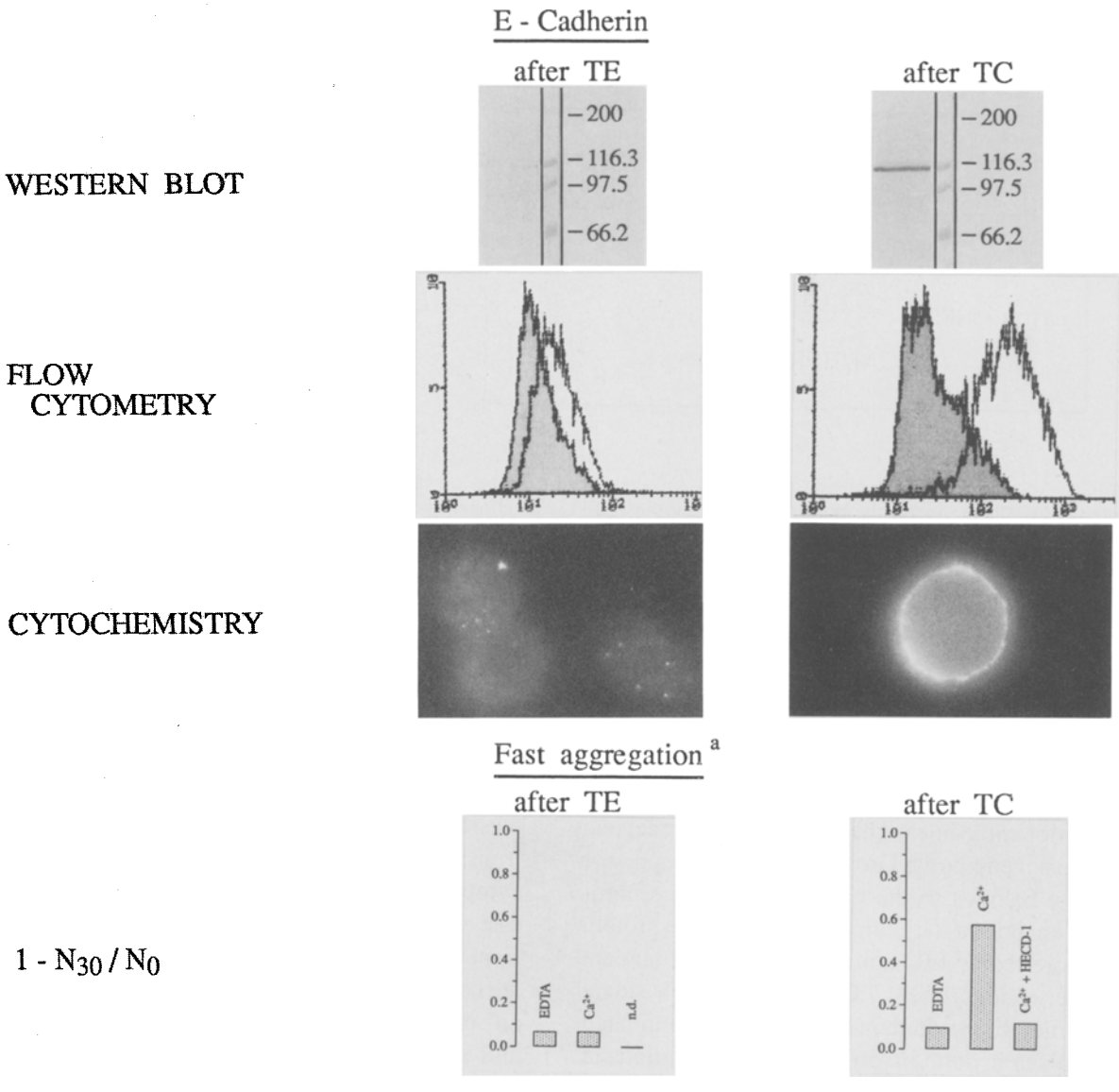


Fig. 3. Schematic representation of cell-cell and cell-substrate adhesion in relationship with the actin cytoskeleton. The window details the molecular domain structure of the 120-kDa functional mature mouse E-cadherin (very high homology in functional domains with human E-cadherin), containing 728 amino acid residues. N, amino-terminus; C, carboxy-terminus; P, an undefined number of phosphorylation sites on Ser and Thr residues; Ca^{2+}, putative calcium-binding domains; flags are potential glycosylation sites; PM, plasma membrane. Modified after [10]; see also [31].

E-cadherin. There are, however, indications that the situation is less simple in as much as cell-cell adhesion molecules may be implicated also in growth. Some tumor suppressor gene products were found to show 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 single transmembrane domain and a cadherin-unrelated cytoplasmic domain [55]. Recessive mutations in the *fat* locus cause loss of single-layered epithelial structure in the larval imaginal discs, as well as tumor formation. Whereas the former effect is dependent upon cell-cell interaction and reminiscent of the anti-invasive function of E-cadherin, the latter is clearly related to growth promotion. The human *DCC* (deleted in colon cancer) gene, a member of the immunoglobulin adhesion molecule family, has been implicated in growth control of the colon mucosa [56]. Recent reports do, however, implicate the inactivation of the *DCC* gene also in the progression from noninvasive to invasive colon carcinoma on the basis of loss of heterozygosity on chromosome 18q including the *DCC* locus [57, 58]. Neither for the *fat* gene product nor for the *DCC* gene products, has it been demonstrated that they actually function as cell-cell adhesion molecules. The cytoplasmic domain of the *fat* gene product is different from that of the cadherins and an intact cadherin cytoplasmic domain is generally thought to be essential for cell-cell interaction. We may, therefore, presume that both types of molecules mediate cell-cell interaction following different mechanisms. A relationship between cell-cell adhesion and growth was also suggested by the experiments of Navarro et al. [59] and of Vleminckx et al. [48]; tumors produced by injection of E-cadherin-positive cells did grow more slowly than tumors from E-cadherin-negative cells. It remains to be examined whether in the above-cited cases alteration of growth results from direct signalling through cadherins, or is an indirect consequence of altered tissue organisation, or is a totally unrelated event.



a. Modified after Kadmon et al. 1990

Fig. 4. E-cadherin-specific fast aggregation of cells prepared by a two-step TE/TC procedure. Stock cultures are rinsed 3 times with Ca^{2+}- and Mg^{2+}-free PBS, followed by a 30-minute incubation with collagenase (0.1 U/ml in Ca^{2+}- and Mg^{2+}-free PBS) and by a 15-minute incubation with trypsin-EDTA (TE). Then, aliquots are taken for E-cadherin expression and fast aggregation (after TE). The rest of the cultures is incubated overnight with fresh culture medium to allow recovery of E-cadherin in preparation of cells for harvesting under E-cadherin-saving conditions. Therefore, cultures are rinsed 3 times with CMF-HBSS (Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution), followed by a 30-minute incubation with collagenase (0.1 U/ml in CMF-HBSS + 0.04 mM $CaCl_2$ + 1 g glucose/l), a 15-minute incubation with TC (0.01% in CMF-HBSS + 0.04 mM $CaCl_2$ + 1 g glucose/l) and addition of soya bean trypsin inhibitor (0.1 U/ml in CMF-HBSS + 1 g glucose/l). Then cells are harvested for E-cadherin expression and fast aggregation (after TC). Immunomanipulations were done with the monoclonal antibody HECD–1. Shaded peaks in flow cytometry represent controls without addition of primary antibody. For fast aggregation measurement numbers of particles were Coulter counted before (N_0) and after (N_{30}) incubation on Gyrotory shaker in accordance with the method of Kadmon et al. [42].

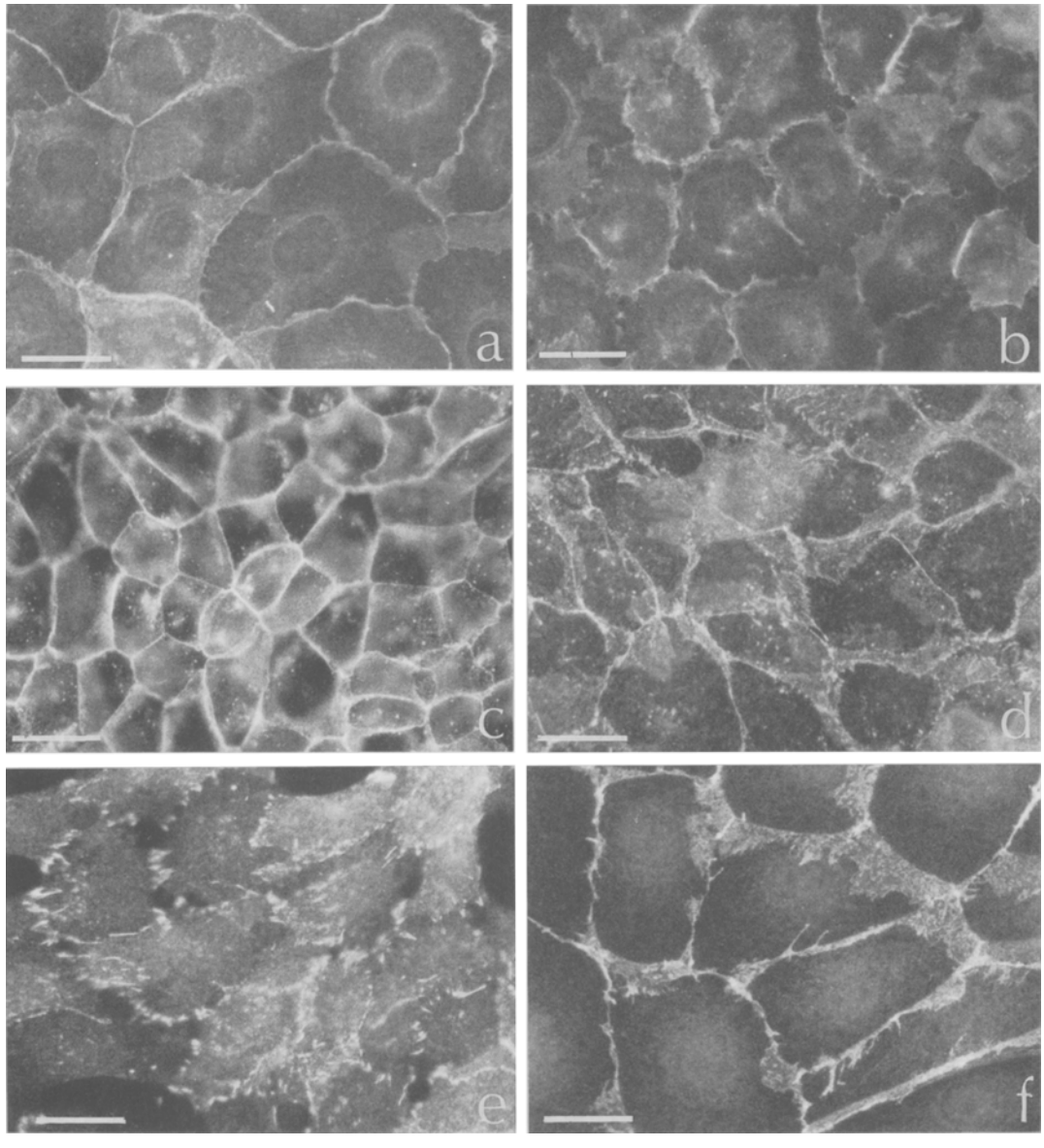


Fig. 5. Different patterns of E-cadherin immunostaining of cells in culture on glass: PROb (a) and REGb (b) rat colon carcinoma cells (unpublished results obtained in collaboration with F. Martin); SH1b1D3 (c), SH1b2D5 (d), and SH1b2B4 (e) canine mammary cancer cells (unpublished results in collaboration with N. Spiekers); MDCK dog kidney cells (f). Scale bars = 20 μm.

E-cadherin expression in development and cancer

During development different cadherins are expressed in a topographically restricted fashion [60]. Amongst them E-cadherin is the most widely distributed and the first to appear during embryogenesis. Synthesis of E-cadherin on mRNA encoded by the embryonic genome starts at the two-cell stage of mammalian development [61]; it becomes phosphorylated at the 8-cell stage, where its distribution is still diffuse. Redistribution of E-cadherin (uvomorulin) from a diffuse to a focalized pattern occurs at the stage of compaction [31]. The role of E-cadherin (uvomorulin) in compaction was demonstrated by inhibition with specific antibodies [62, 63]. Dur-

ing avian gastrulation, cells of the upper layer lose their coherence and ingress through the primitive streak. Edelman et al. [64] showed immunohistochemically that L-CAM (synonym for chicken E-cadherin) was present between the cells of the upper layer in the groove of the primitive streak of the chick blastoderm at stage 9 of Vakaet [65]. Upper layer cells ingress and form the middle layer, a phenomenon comparable with cancer cell invasion. Thereafter, loss of cell-cell adhesion leads to detachment of the middle layer cells and this phenomenon is accompanied by loss of the expression of L-CAM. During organogenesis, cell-cell adhesion may be reinstalled in some tissues. For example, Vestweber et al. [66] found that uvomorulin (E-cadherin) was present in kidney tubules that had recently (12 h) been formed out of uvomorulin-negative mesenchyme. The authors did not succeed in preventing formation of tubules by anti-uvomorulin antibodies, although they demonstrated that their 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. An alternative explanation is that the antibodies had no neutralizing effect despite their proper binding, as was observed by us with another antibody. Desmosomal proteins appear later during development, namely at morula to-early-blastocyst stages, with the exception of plakoglobin which is synthezised at the compaction stage and becomes membrane-accentuated during late morula [67]. It is interesting to recall that plakoglobin belongs to the catenin family of E-cadherin-associated proteins. In the human placenta expression of E-cadherin is high in the villous cytotrophoblast; during differentiation and invasion the expression decreases [68, 69]. Maximal increase of E-cadherin as evident from Western blots coincided with maximal aggregate formation between cytotrophoblast cells in culture.

Although E-cadherin is expressed mostly by epithelial cells in both embryon and adult, exceptions have been described. Particular regions of chick sensory ganglia and brain [70] and Langerhans' cells of the adult mouse skin [52] are E-cadherin-positive. The latter finding is quite unexpected since Langerhans' cells are antigen presenting cells that migrate from the bone marrow to the skin and from the skin to the lymph nodes. In adult tissues there is an event during which the compaction-specific redistribution of E-cadherin is reversed, namely mitosis. Indeed, in all M-phase cells the distribution is diffuse over the cell surface [see Fig. 2 in ref. 71].

Mouse skin tumors induced by a two-stage chemical carcinogenesis protocol show a reduced expression of uvomorulin and of Na^+, K^+-ATPase when they progress from the benign to the malignant stage [72]. The first paper indicating alterations of the expression of E-cadherin in a variety of human tumors was published by Eidelman et al. [73]. During the last three years more detailed reports have appeared about most human cancers (Table 1). The general picture emerging from these papers is that in the epithelia of origin and in benign tumors E-cadherin is expressed by all cells (homogeneity) and that it is localized at the cell-cell contacts; in cancers, E-cadherin expression is heterogeneous and its location is more diffuse (for example, see Fig. 6). Such alterations are most obvious in poorly differentiated tumors which are known to be the more aggressive ones. Few studies have investigated the relevance of disturbed E-cadherin expression for clinical malignancy. Immunohistochemistry of more than 100 gastric cancers showed a correlation of reduced E-cadherin expression with local invasion and metastasis [74]. However, no correlation was found between E-cadherin-positivity and Dukes' stage in colon cancer [75].

Taken together these data show alterations in E-cadherin expression in malignant, i.e., invasive cancers, and this is in agreement with the idea that E-cadherin is an invasion-suppressor molecule [10, 76]. However, the static observations on human biopsy specimens do not prove that expression of E-cadherin is the key event in epithelial organisation (I-minus phenotype) or that downregulation of E-cadherin is initiating malignancy (I-plus phenotype). Clearly, experimental evidence is needed to support or to refute these statements.

Table 1. Aspects of E-cadherin expression in human tumors

Type of tumor	E-cadherin		References
	Method*	Aspect	
Breast	RFLP Immunohistochemistry (HECD-1)	LOH 16 q Abnormally localised; reduced	[19, 121, 122]
Lung	Immunohistochemistry (HECD-1)	Present	[123]
Colon	Immunohistochemistry (DECMA-1; 6F9) *In situ* hybridization		[75, 124, 125]
Prostate	RFLP *In situ* hybridization	allelic loss reduced to absent	[89, 126]
Head and neck	Immunohistochemistry (6F9) *in situ* hybridization	reduced to absent	[127]
Esophagus & stomach	Immunohistochemistry (HECD-1)	Abnormally localised; reduced	[74,128]
Female genitalia	Immunohistochemistry (HECD-1)	Abnormally localised; reduced	[129]
Neurectodermal	RFLP	LOH 16q	[90]
Meningioma	Immunohistochemistry (HECD-1)	abnormally localised	[130]
Skin	Immunohistochemistry (not mentioned)	reduced	[131]
Wilms'	RFLP	LOH 16q	[93]
Hepatocellular	RFLP	LOH 16q	[92]
Miscellany	Immunohistochemistry	Abnormally localised; reduced	[73]

* Type of antibody in parenthesis

E-cadherin has been looked at also in a few noncancerous conditions.

In Darier disease with hyperkeratosis and crusted papules of the skin a markedly reduced immunofluorescence (HECD–1) was found in sites of acantholysis [77]. In murine polycystic kidney disease (*cpk/cpk* mice), characterized by phenotypically disturbed epithelium of tubules and ducts with dysfunctional transport, kidney-specific downregulation of both E-cadherin and N-CAM was an early event, as evidenced by Northern blotting and immunostaining [78]. Immediately after wounding of the mouse corneal epithelium, immunostaining demonstrated a redistribution of the actin-binding protein

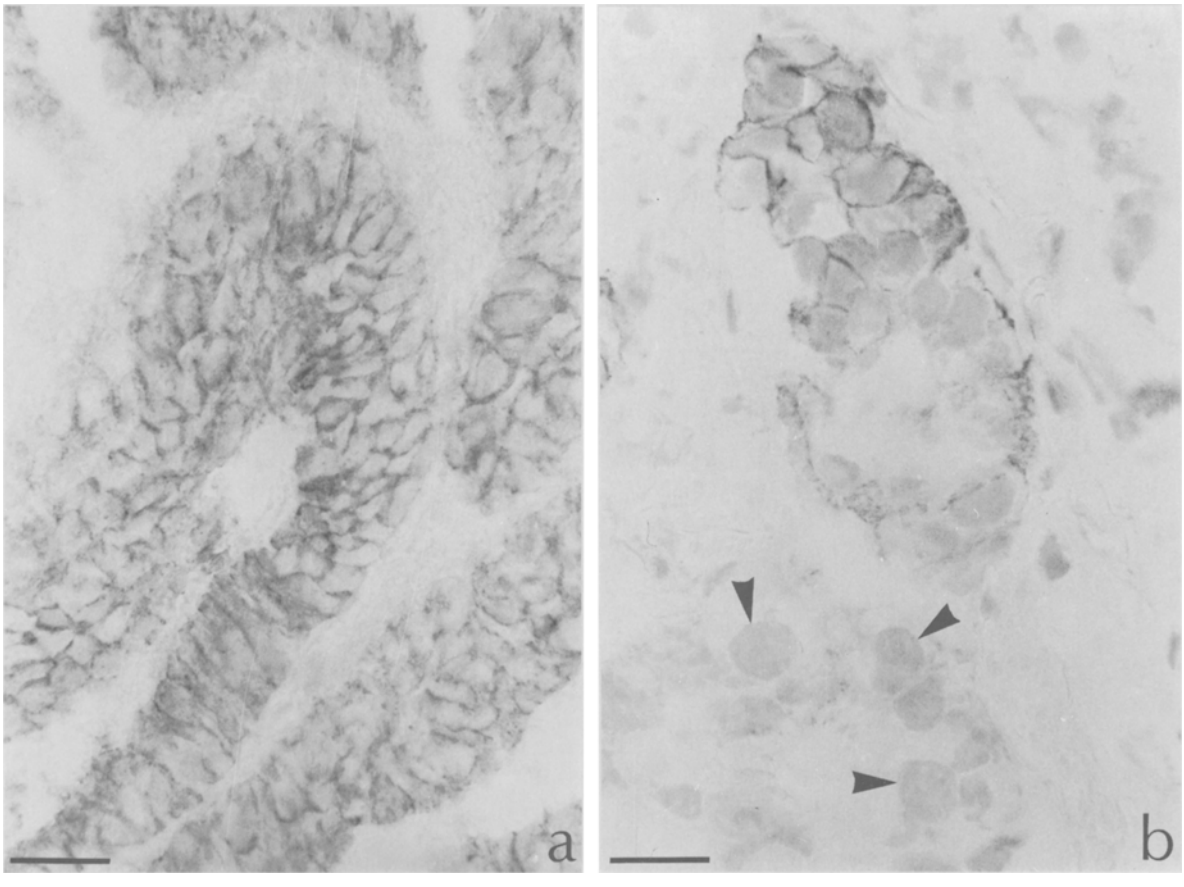


Fig. 6. Photomicrographs of frozen sections from a well differentiated (a) and from a poorly differentiated (b) human colon cancer; immunostaining with the E-cadherin-specific monoclonal antibody MLCA (Euro-Diagnostics; Apeldoorn, The Netherlands); arrowheads indicate E-cadherin-negative cancer cells inside the stroma and identified by immunostaining for cytokeratin (not shown); Scale bars = 20 μm (unpublished results in collaboration with J. Van Aken).

fodrin throughout the cytoplasm, but the honeycomb pattern of E-cadherin did not change [79].

Experiments in vitro

Experimental manipulations *in vitro* that have provided arguments in favour of an invasion-suppressor role of E-cadherin in various epithelium-derived cell types have been reviewed by Van Roy and Mareel [10], by Mareel et al. [49] and by Behrens et al. [76]. Transition of mesenchymal (fusiform or f-type) cells to epithelial (epithelioid or e-type) cells after transfection with E-cadherin suggests that E-cadherin may initiate the organization of an epithelium (I-minus phenotype). Indeed, in the experiments by McNeill et al. [80] E-cadherin-mediated aggregation was followed by redistribution of cell surface-associated molecules like Na^+, K^+-ATPase and installation of vectorial transport functions. This process was Ca^{2+}-dependent and necessitated intact E-cadherin bound homophylically to E-cadherin on another cell and linked with the cytoskeleton at its cytoplasmic domain. For several families of animal cell lines and for a variety of human cancer cell lines a negative correlation was found between the expression of E-cadherin and invasion *in vitro* [47, 48, 81, 82]. In most of these experiments the expression of E-cadherin was demonstrated in culture on solid tissue culture substrate, i.e., in a micro-ecosystem that may differ much from the one established in the

invasion assays used. In other experiments E-cadherin expression was examined immunohistochemically under the conditions of the invasion assay [83]. When human trophoblast cells were seeded on Matrigel, E-cadherin was found to be lacking specifically at sites where active invasion was suspected [68]. *In vitro*, transitions from the I-plus to the I-minus and from the I-minus to the I-plus phenotype were possible by cDNA transfection. In cancer cell types that were E-cadherin-negative, invasion could be abrogated by transfection and expression of exogenous E-cadherin cDNA [48, 81, 82]. In E-cadherin-positive, invasion-negative cells, reduction of E-cadherin mRNA levels after transfection with a plasmid expressing E-cadherin-specific antisense RNA induced the invasive phenotype [48]. Invasion could also be induced by immunoneutralization in cells expressing the endogenous or the exogenous E-cadherin at high and homogeneous levels. Note that not all antibodies are functionally inactivating despite their specific binding and that the antigen cannot always be reached by the antibody. Absence of E-cadherin does not necessarily implicate the expression of the I-plus phenotype. 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* [48]. The invasive behaviour of these cells was suppressed by transfection with E-cadherin cDNA, despite unchanged high levels of *ras* expression, indicating that E-cadherin may counterbalance the invasion promotor activity of the *ras* oncogene. How such oncogene induces the expression of the invasive phenotype and at what level this is counterbalanced by E-cadherin is an open question [10, 84].

Retention of Langerhans' cells in the skin can be considered as a normal type of metastasis that can be compared to some extent with dermotropic lymphoma [1]. In both cases circulating cells home to the epidermis and are retained there for some time. Surprisingly, freshly prepared mouse Langerhans' cells were found to be E-cadherin-positive; they did adhere to keratinocytes in monolayer culture but not to E-cadherin-negative L cells [52]. The adhesion was Ca^{2+}-dependent and sensitive to antibodies against E-cadherin. Cultured Langerhans' cells, which are thought to be equivalents of Langerhans' cells that have migrated to lymph nodes, expressed lower levels of E-cadherin and adhered poorly to keratinocytes. It would be of interest to know whether in the *in vitro* adhesion experiments Langerhans' cells adhere to the apical surface of the monolayer cells or are inserted between these cells. Furthermore, one would like to see an histochemical demonstration of E-cadherin on Langerhans' cells in the epidermis *in vivo*.

E-cadherin expression: in vitro *as compared to* in vivo

For selected cell lines, comparison between E-cadherin expression *in vitro* and *in vivo* provided a first experimental indication of sensitivity to micro-environmental influences. Indeed, injection into nude mice of homogeneously E-cadherin-positive cell types led to formation of tumors which were heterogeneous with regard to E-cadherin expression. Interestingly, whereas these type of cells were noninvasive *in vitro*, the tumors were invasive and sometimes also metastatic [5, 85]. Suppression did occur with both endogenous and transfected E-cadherin, despite the fact that the latter was under the control of a supposedly constitutive promoter [48, 86]. Zonal distribution of E-cadherin-positive and negative cells may be explained as clonal expansion of a progenitor cell in which E-cadherin expression was lost or as zonal distribution of downregulating factors. Selection in the host animal for rare and stable E-cadherin-negative variants is unlikely, because of the ready restoration of expression upon *ex vivo* culture. Furthermore, E-cadherin appears to be re-expressed in larger metastases, although these secondary tumors never reach the fraction of positive cells observed in the primary tumor (Fig. 7). We have concluded from these *in-vitro/in-vivo/ex-vivo* observations that the loss of E-cadherin in tumors is temporary. As put forward also by Gabbert et al. [87], such transient loss would allow invasion and it would last long enough for metastasis. The def-

inition of a metastatic cancer would then be: a cancer in which at least part of the cell population does invade for a certain period of time. This activity may stop and resume later on.

Levels of E-cadherin regulation

Cadherins are localized at the cell surface (Fig. 8). They are plasma membrane receptors, the structure of which permits regulation at various levels (see Fig. 3).

Mutant E-cadherins have been expressed in cultured cells [88], but in natural tumors no mutations have been found for cadherins, so far. In cancers, loss of heterozygosity (LOH) at specific chromosomal locations is accepted as an indication for a tumor suppressor gene at these locations. In prostate cancer, LOH at chromosome 16q was a frequent (30%) event [89]. LOH at 16q was found also in 3 out of 25 tumors from the neurectoderm [90], whereas in breast cancer, allele loss on 16q correlated positively with lymph node metastases [91]. In hepatocellular carcinomas, a tumor suppressor gene has been localised on chromosome 16q22.1 to 16q23.2, i.e., the region where the E-cadherin gene is located [92]. The late loss of this allele during hepatocarcinogenesis is consistent with an invasion suppressor role of E-cadherin in these tumors. In 20 % of sporadic Wilms' tumors (WT), Huff et al. [93] did find LOH at 16q13–16q22 that did not seggregate in WT families but was tentatively implicated in tumor progression [94].

DNA sequences sensitive to tissue specific regulatory elements have been identified in the E-cadherin promoter [76]; in a CAT (Chloramphenicol Acetyl Transferase) reporter assay, these sequences were found to be highly active in differentiated breast carcinoma cells but silent in undifferentiated carcinoma cells. Genomic alterations in tumor cells are rarely unique. They are usually associated with multiple changes of the same or of other chromosomes. This makes it difficult to establish causal relationships between chromosomal changes and tumor phenotypes; it has even been proposed that the accumulation of allelic deletions is more important than the order in which they occur [56]. Dominant mutants lacking E-cadherin expression but not showing gross defects in the E-cadherin gene have been derived from compaction-competent embryonal carcinoma cell lines [95]. These mutants have lost compaction-competence and may be useful tools to study developmentally regulated expression of cadherin by trans-acting factors.

Transcriptional downregulation of E-cadherin has been documented in several cases. Such a downregulation coincided with fusion of human cytotrophoblast *in vitro*, as evident from Northern blots [69].

In the mouse skin tumors, described by Ruggeri et al. [72], mRNA levels were in line with immunohistochemical signals. In higher grade squamous cell carcinomas of the mouse skin the E-cadherin mRNA level was marginal or undetectable. Downregulation of exogenous E-cadherin was shown to occur also at the transcriptional level [86]. Transfection with E-cadherin cDNA of mouse MO_4 cells from mesenchymal origin resulted in the isolation of MO_4 cells, that did express exogenous E-cadherin at their surface. These transfectants, in contrast with their parental cells, showed Ca^{2+}-dependent fast aggregation and failed to invade into collagen type 1. When such 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. Northern blotting revealed levels of E-cadherin mRNA that were 10 times lower in the tumors than in the cells that were injected. *Ex vivo* culture of these tumors led to rapid re-expression of E-cadherin, indicating downregulation of E-cadherin at the transcriptional level under the influence of host factors. Temporary downregulation of E-cadherin in invasive rat prostate cancers with re-expression in some parts of the metastases occurred also at the transcriptional level as evidenced by Northern blot analysis [96]. The situation in human cancers as revealed by *in situ* hybridization is summarized in Table 1.

Posttranslational modulation of E-cadherin, with or without alteration of the immunosignal, is an alternative to transcriptional regulation and can

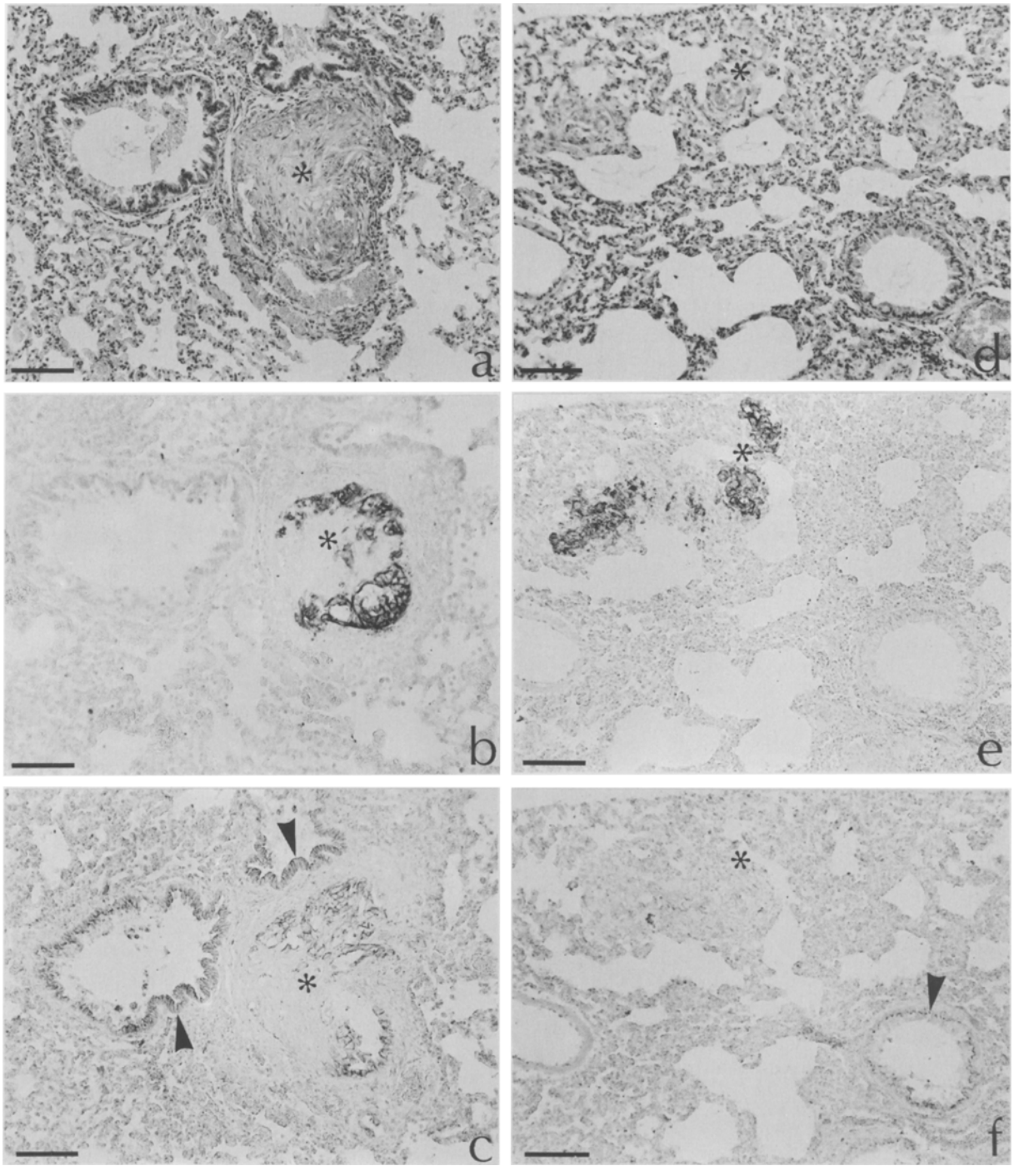


Fig. 7. Photomicrographs of paraffin sections from a larger (a to c) and a smaller (d to f) metastasis (asterisks) in the lungs of a nude mouse bearing a subcutaneous tumor after injection of MDCK cells from an *in vitro* culture that was homogeneously positive for E-cadherin; consecutive sections were stained with hematoxylin and eosin (a and d), with an antibody against MDCK cells (b and e), and with antibody α-UMT [47] against E-cadherin (c and f); arrowheads point to E-cadherin staining of bronchioli. Scale bars = 100 μm.

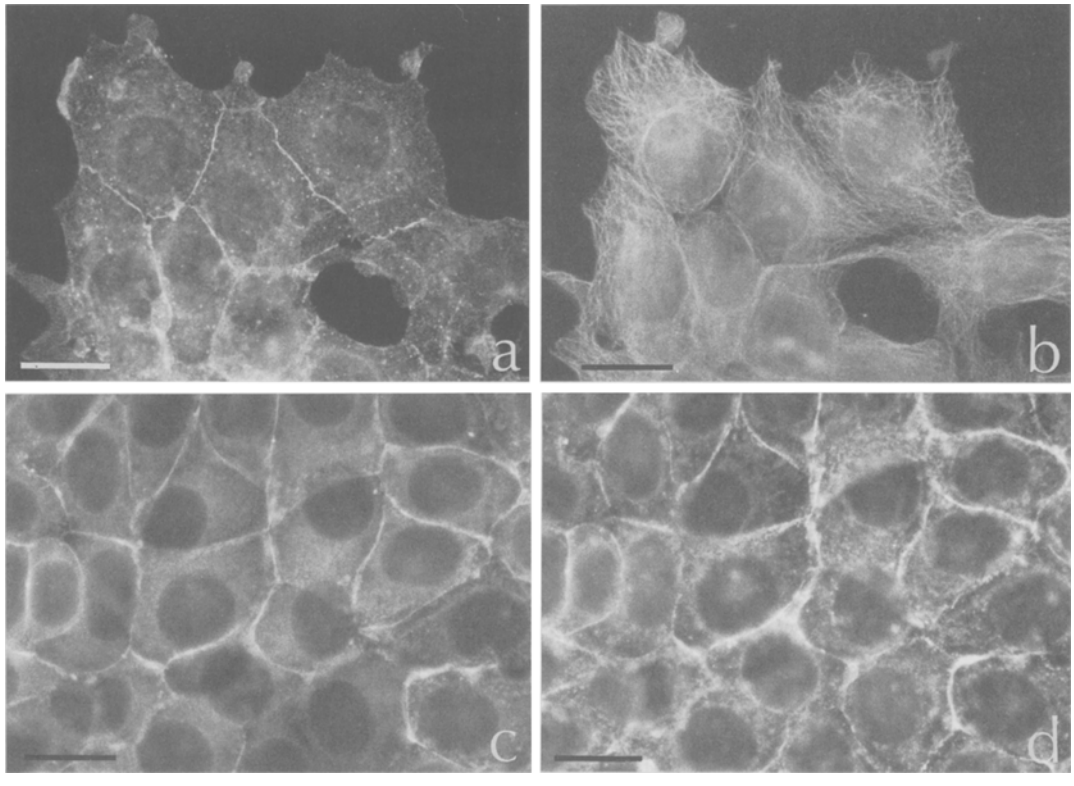


Fig. 8. Double immunostainings of human MCF-7/AZ (a and b) and MCF-7/6 (c and d) mammary cancer cells in culture with anti-E-cadherin (a) or anti-tubulin (b) antibodies, and with anti-E-cadherin (c) or anti-phosphotyrosine (d) antibodies; note that microtubules terminate at the plasma membrane mainly in E-cadherin-negative areas and that E-cadherin colocalizes with phosphotyrosine at the cell-cell border. Scale bars = 20 μm.

occur at various levels (see Fig. 3). Protein degradation is one possibility. During the early phase of polarity development in dog kidney MDCK cells spatial restriction of desmoglein-I and of E-cadherin is not due to selective routing of these molecules to the lateral surface but to their rapid removal from the apical cell surface by internalisation and subsequent degradation [97]. Complex formation with other proteins (other E-cadherins and catenin/actin complexes) at sites of cell-cell contact would prevent such rapid degradation. For N-cadherin it was demonstrated that its loss from the developing retina was inhibited *in vitro* by the metalloprotease inhibitor 1,10 phenantroline [98].

Since E-cadherin is a glycoprotein of the complex type, glycosylation may well be a way of fine tuning. 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 [33]. The role of the carbohydrate residues in the homophilic adhesion or the signalling function of E-cadherin largely remains to be explored.

Ca^{2+} ions are needed for the stabilization of cadherins and for their correct assembly into adherens junction complexes. Since extracellular Ca^{2+} levels are known to vary within tissues, this provides another possible mechanism of regulation of cadherin [34].

Lack of binding to catenins results in loss of the cell-cell adhesion function of E-cadherin [37, 99, 88]. Recent work with *Xenopus* embryos indicated that the cytoplasmic domains of different cadherins may compete for binding to catenins [100]. During early cleavage in *Xenopus*, the animal pole ectoderm produces epidermal epithelium and neuroepithelium expressing E-cadherin and N-cadherin, respectively [101–106]. Injec-

tion 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 [107]. The same result was obtained with a mutant form of N-cadherin, designated 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. Human lung carcinoma PC9 cells, expressing E-cadherin all around their surface and β-catenin in their cytoplasm but neither αE-catenin nor αN-catenin, grew as isolated cells suggesting deficient E-cadherin function [108]. After transfection with αN-cadherin cDNA these cells formed aggregates showing epithelial and sometimes also cystic organisation. The authors concluded that α-catenin was crucial not only for E-cadherin-mediated cell-cell adhesion but also for the organisation of multicellular structures. How the E-cadherin/catenin complex fulfils this task is hardly understood. Immunocytochemistry and immunodetection on Western blots from total extracts of PC9 cells were negative for α-catenin, whereas Northern blots showed a low α-catenin-specific signal at the level of a smaller-than-normal mRNA fragment [43]. Southern analysis with a cDNA probe covering the entire open reading frame suggested an homozygous deletion of part of the α-catenin gene. The authors mentioned data in preparation showing that in E-cadherin-positive, yet aggressive, stomach cancers with 'scattered phenotype' α-catenin is lacking.

The carboxyterminal half of the cytoplasmic domain of E-cadherin is essential for catenin binding and carries multiple putative phosphorylation sites. The dynamic balance between phosphorylation and dephosphorylation of tyrosine or threonine/serine residues provides an interesting tool for functional regulation and this is sustained by experimental observations. Protein kinase C has been found to trigger premature compaction of mouse embryos [34] and there are indications that increased tyrosine phosphorylation directly or indirectly prevents formation of adherence junctions [109]. Although these observations suggest an implication of E-cadherin, direct evidence for functional regulation of E-cadherin *via* phosphorylation has been provided only recently. Madin-Darby canine kidney (MDCK) epithelial cells transformed with a temperature-sensitive mutant of *v-src* exhibit an epithelial morphotype at the nonpermissive temperature (40 °C) [110]. After culture at the permissive temperature (35 °C) $pp60^{src}$ is active, cell-cell contact and transepithelial electrical resistance are lost and cells become invasive into collagen gel and into chick heart *in vitro*. These effects were linked to an increase in tyrosine phosphorylation of E-cadherin and in particular of β-catenin. The role of tyrosine phosphorylation is confirmed by the observation that a potent inhibitor of tyrosine kinases, namely herbimycin A prevents the morphotypic transformation (Fig. 9) and inhibits invasion at permissive temperature (unpublished observations in collaboration with L. Vakaet, Jr). A novel tyrosine kinase substrate, implicated in cell transformation by $p60^{v-src}$, was shown to contain repeated segments that occur also in the protein encoded by the (40 °C) *armadillo* gene of *Drosophila* and in its homologues plakoglobin and β-catenin [111]. Rat cells transformed with *v-src* were unable to retain stable cell-cell association despite the expression of endogenous P-cadherin and exogenous E-cadherin [44]. In such cells tyrosine phosphorylation of a 89-kDa catenin (presumably β-catenin) and also to a lower extent of cadherin perturbed cadherin function. In chick embryo cells transformed with temperature sensitive mutants or with non-myristylation mutants of *v-src* tyrosine phosphorylation of N-cadherin and catenin suppressed cell-cell adhesion in a transformation-specific way [45]. Treatment of *v-src*-transformed cells with herbimycin A inhibited phosphorylation of cadherin and catenins and concomitantly led to recovery of aggregation-competence to the level of untransformed cells. The *src* oncogene product $pp60^{src}$ not only influ-

ences cell-cell adhesion but is also implicated in cell substrate adhesion e.g., through interaction with the focal-adhesion-associated protein kinase $pp125^{FAK}$ [112].

Regulators of E-cadherin expression and function

We have only started to learn something about the environmental factors that influence the expression and function of E-cadherin at the various levels discussed above. Our *in vivo* experiments with E-cadherin-positive cells [85] demonstrate an easy way to establish a micro-ecosytem for downregulation, but the elements seem to be multiple and poorly defined. Downregulatory factors meeting stringent criteria could not be identified, so far. Conditioned medium from a human skin cancer cell line caused scattering of MCF-7 cells and interfered with their Ca^{2+}-dependent fast aggregation (unpublished results in collaboration with T. Boterberg). Candidate factors are IL–1 (our unpublished results) and TGF-β [6], both of which induce some changes in the distribution of E-cadherin in some types of cells in culture. NBT-II rat bladder cells, treated with acidic fibroblast growth factor (aFGF) underwent an epithelioid-to-fusiform (e-to-f) transformation with redistribution of E-cadherin over the entire cell surface [113]. However, the authors think that the scattering activity of aFGF is not mediated by direct modulation of E-cadherin.

Cadmium at non-toxic concentrations decreased the amount of surface exposed E-cadherin and reduced the transepithelial electrical resistance in epithelial cells in culture, possibly by interacting with the Ca^{2+}-binding domains [114]. Although cadmium is an improbable candidate for inducing *in vivo* loss of E-cadherin function, this observation points again to the importance of the Ca^{2+}-binding domains. Finally, insulin has been found to enhance downregulation (mRNA level) of N-cadherin during development of the retina [98].

What about structurally or functionally upregulating factors? In the rat epididymis, maintenance of E-cadherin mRNA levels depends on testosteron as evident from orchiectomy followed or not by replacement with testosterone [115]. A hormonal regulation of E-cadherin has recently been found by us with a cell line that was E-cadherin-positive, yet scored negative in the functional assays (unpublished results). A remarkable example was found in the MCF-7 human breast cancer cell family, where we have at our disposal closely related variants (Fig. 10) that show discrete differences in phenotypes of interest. One of the rare invasive variants, coined MCF-7/6 [116], appeared to be E-cadherin-positive upon immunostaining of cells in culture with monoclonal antibody MLCA (Euro-Diagnostics, Apeldoorn, The Netherlands). Flow cytometry demonstrated the presence of E-cadherin on the cell surface (Fig. 11a). Western blots of total MCF-7/6 cell extracts with different monoclonal antibodies against human E-cadherin, revealed a 120-kDa band, which corresponds to the human E-cadherin molecule (Fig. 11b). When islands of MCF-7/6 cells, cultured on glass, were stained immunocytochemically with a monoclonal antibody against E-cadherin, a typical honeycomb pattern was revealed, showing expression of E-cadherin at cell-cell contact sites (Fig. 11c). Moreover, E-cadherin was demonstrated in cryosections from chick heart with a positive signal at the surface of MCF-7 cells, even in the case of cells that had invaded the host tissue. We wondered why E-cadherin was unable to exert its invasion-suppressor role in MCF-7/6 cells within the chick heart organ culture micro-ecosystem. Therefore, we tried to make E-cadherin functional by adding a number of external factors to the culture medium. Since the organ culture assay takes too much time for screening purposes, the aggregation assays were used for this purpose (see Fig. 4). MCF-7/6 cells showed little tendency to aggregate. Insulin-like growth factor-I (IGF-I) clearly increased aggregation and this effect was mimicked by high doses (1 μg/ml) of insulin, but not by IGF-II (up to 0.5 μg/ml) or by EGF (100 ng/ml). The IGF-I effect on aggregation is receptor-dependent since it is counteracted by the IGF-I-specific monoclonal antibody αIR3. This is in line with our observations that IGF-I-induced fast aggregation is inhibited by several tyrosine kinase inhibitors (our unpublished results). The E-

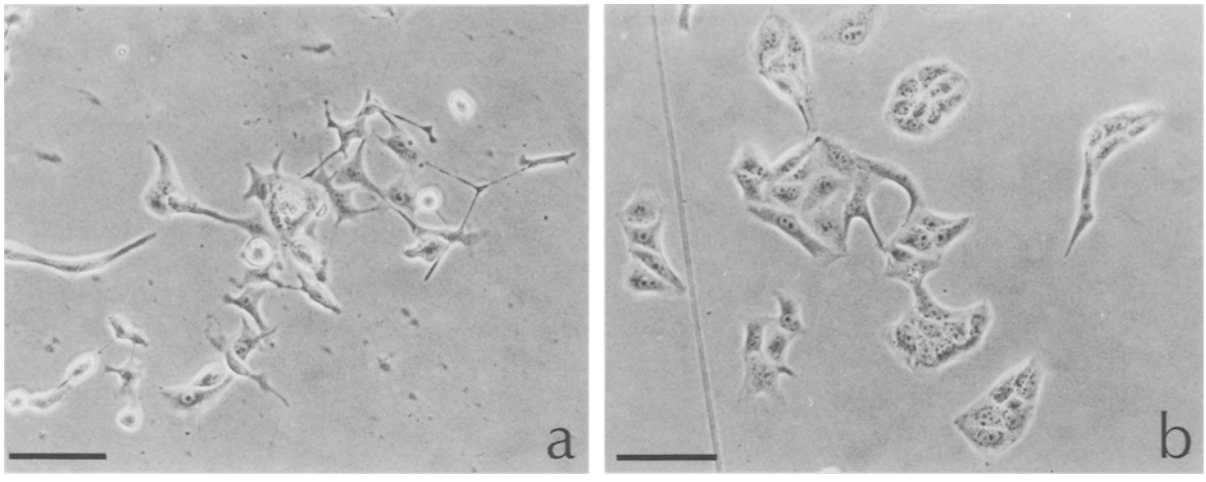


Fig. 9. Morphotype of MDCK cells transformed by a temperature sensitive mutant of *v-src* in culture on solid substrate at the transformation-permissive temperature of 34 °C without (a) and with (b) herbimycin A at 0.01 μg/ml; scale bars = 20 μm (from unpublished results in collaboration with L. Vakaet Jr).

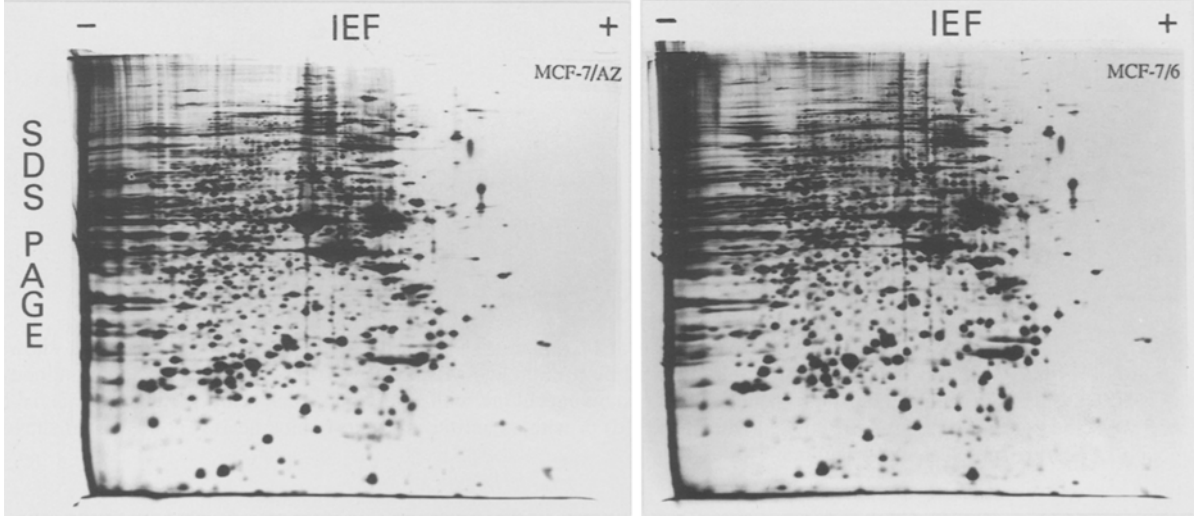


Fig. 10. Close relationship between noninvasive (MCF-7/AZ) and invasive (MCF-7/6) cells as evident from the distribution of total cell proteins after two-dimensional separation in accordance with the method of O'Farrell [119]. Equal amounts of proteins in lysis buffer were applied to isoelectric focusing gels (pH 3 to 8). 10% slab gels were used for SDS PAGE in the second dimension. The gels were silver-stained in accordance with Heukeshoven and Dernick [120].

cadherin-dependence of the reaction was demonstrated by inhibition with a monoclonal antibody specific for E-cadherin. IGF-I-induced fast aggregation was not dependent upon *de novo* protein synthesis as evident from treatment with cycloheximide. Remarkably, invasion of MCF-7 cells into chick heart was inhibited by addition of IGF-I to the culture medium and this effect was also neutralized by the IGF-I receptor-specific αIR3 antibody. We are currently investigating the relationship between the tyrosine kinase receptor for IGF-I and the E-cadherin/catenin/actin complex. It also remains to be explained how our data on anti-invasiveness of IGF-I fit with the role of this molecule as a positive growth factor and as a marker of poor prognosis in human breast cancer [117]. One general point to recall here is that most growth factors are multifunctional in a cell type-defined way [12]. So, IGF-I has been shown

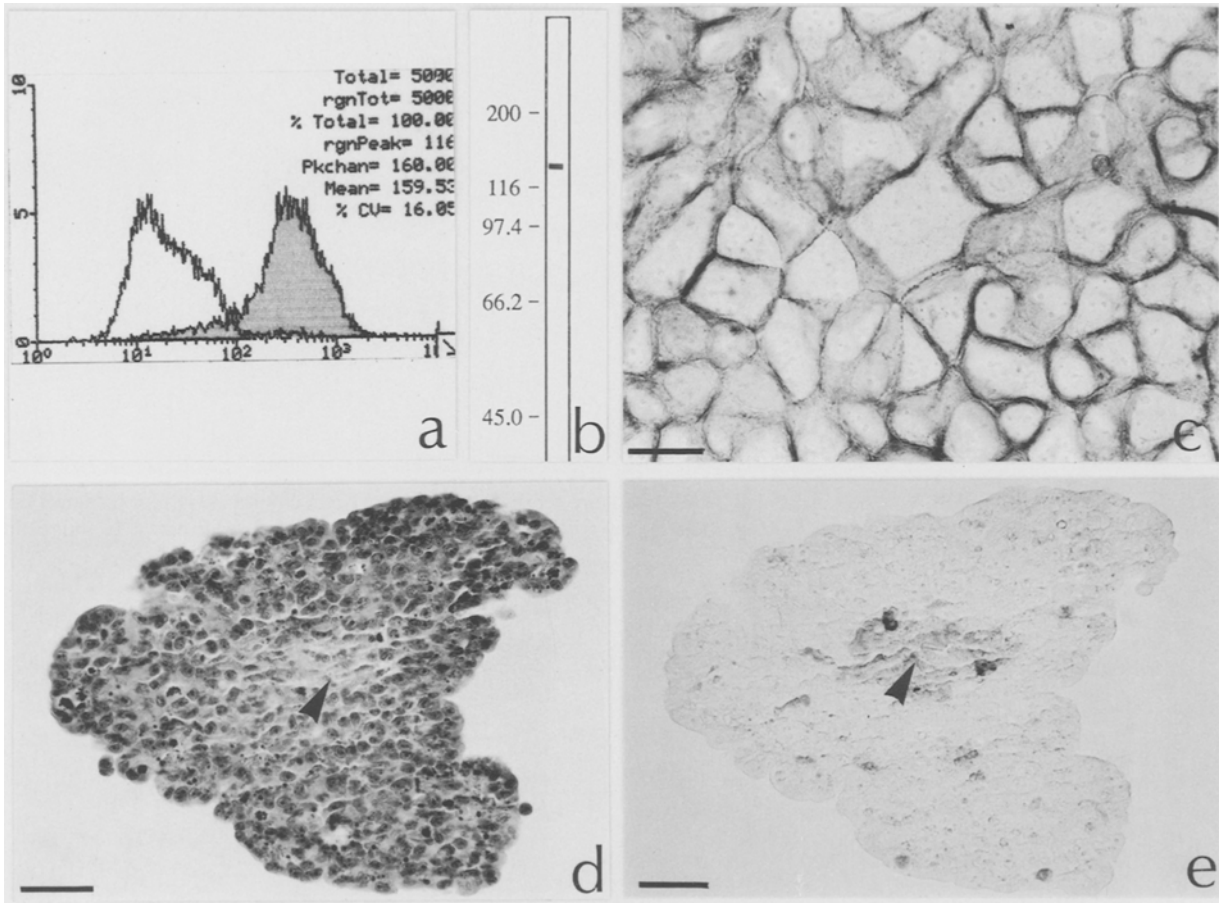


Fig. 11. Characterization of MCF-7/6 cells. Expression of E-cadherin is shown by (a) flow cytometry (first peak shows omission of primary antibody), by (b) Western blotting and by (c) immunocytochemistry; the primary monoclonal antibody used is HECD–1. Expression of the I-plus phenotype in organotypic culture with embryonic chick heart; the culture was fixed after 8 days and paraffin sections were stained with H & E (d) or with an antiserum against chick heart (e); arrowhead shows remnants of heart tissue. Scale bars = 50 mm.

to promote differentiation of human colon carcinoma cells *in vitro* [118].

Conclusion

Metastasis determines cancer malignancy. Neoplastic cells metastasize through a multistep process of invasion. At each step, these cells create a dynamic micro-ecosystem in which the elements of the host are considered to paricipate actively invasion. Within such micro-ecosystems, invasion is believed to be governed by a balance between the activation of promoter (*i*-minus) and suppressor (*i*-plus) genes. Products of such genes regulate the expression of the invasive (I-plus) and the noninvasive (I-minus) phenotypes. Experiments *in vitro* and *in vivo*, as well as observations on human cancers have put forward the cell-cell adhesion molecule E-cadherin (L-CAM; uvomorulin) as a regulator of epithelial organization and an invasion suppressor.

Acknowledgements

Research in the authors' laboratories is supported by the Fonds voor Geneeskundig Wetenschappelijk Onderzoek (FGWO), the Algemene Spaar- en Lijfrentekas (ASLK), Kom Op Tegen Kanker,

Levenslijn, the Sport Vereniging Tegen Kanker, The Department of Citrus, State of Florida, USA, the Onderzoeksfonds RUG, Belgisch Werk Tegen Kanker, Centrum voor Gezwelziekten, Nationale Loterij and the Vereniging voor Kankerbestrijding. F.V.R. is Research Director with the N.F.W.O. Belgium.

References

1. Mareel MM, De Baetselier P & Van Roy FM (1991) Mechanisms of invasion and metastasis. CRC Press, Boca Raton/Ann Arbor/Boston
2. Mareel MM & Crombez R (1992) Acta Oto-Rhino-Laryngol. 46: 107–115
3. Fidler IJ (1990) Cancer Res. 50: 6130–6138
4. Mareel M, Van Roy F & De Baetselier P (1990) Cancer Metastasis Rev. 9: 45–62
5. Mareel M, Vleminckx K, Gao Y, Van Larebeke N, Bracke M & Van Roy F (1992) In: Rabes H, Peters PE & Munk K (Eds) Metastasis: Basic research and its clinical applications vol 44 (pp 141–153) Contrib. Oncol. Karger, Basel
6. Van Roy F, Vleminckx K, Vakaet L Jr, Berx G, Fiers W & Mareel M (1992) In: Rabes H, Peters PE & Munk K (Eds) Metastasis: Basic research and its clinical applications vol 44 (pp 108–126) Contrib. Oncol. Karger, Basel
7. Vogelstein B & Kinzler KW (1992) Cell 70: 523–526
8. Nicolson GL (1991) Semin. Cancer Biol. 2: 143–154
9. Radinsky R & Fidler IJ (1992) In Vivo 6: 325–332
10. Van Roy F & Mareel M (1992) Trends in Cell Biology 2: 163–169
11. Lotan R, Amos B, Watanabe H & Raz A (1992) Cancer Res. 52: 4878–4884
12. Gherardi E & Stoker M (1991) Cancer Cells 3: 227–232
13. Weidner KM, Behrens J, Vandekerckhove J & Birchmeier W (1990) J. Cell Biol. 111: 2097–2108
14. Mareel M, Bracke M, Gao Y & Van Roy F (1991) Eur. Arch. Biol. 102: 185–188
15. Tsarfaty I, Resau JH, Rulong S, Keydar I, Faletto DL & Vande Woude GF (1992) Science 257: 1258–1261
16. Liotta LA, Steeg PS & Stetler-Stevenson WG (1991) Cell 64: 327–336
17. Correc P, Fondanèche M-C, Bracke M & Burtin P (1990) Int. J. Cancer 46: 745–750
18. Basset P, Bellocq JP, Wolf C, Stoll I, Hutin P, Limacher JM, Podhajcer OL, Chenard MP, Rio MC & Chambon P (1990) Nature 348: 699–704
19. Foekens JA, Schmitt M, Van Putten WLJ, Peters HA, Bontenbal M, Jänicke F & Klijn JGM (1992) Cancer Res. 52: 6101–6105
20. Garbisa S, Scagliotti G, Masiero L, DiFrancesco C, Caenazzo C, Onisto M, Micela M, Stetler-Stevenson WG & Liotta LA (1992) Cancer Res. 52: 4548–4549
21. Mareel MM, Van Roy FM & Bracke ME. Crit. Rev. Oncogenesis (in press)
22. Van Waes C, Kozarsky KF, Warren AB, Kidd L, Paugh D, Liebert M & Carey TE (1991) Cancer Res. 51: 2395–2404
23. Hart IR, Birch M & Marshall JF (1991) Cancer Metastasis Rev. 10: 115–128
24. Johnson JP (1991) Cancer Metastasis Rev. 10: 11–22
25. Roos E (1991) Cancer Metastasis Rev. 10: 33–48
26. Hynes RO & Lander AD (1992) Cell 68: 303
27. Tanaka Y, Adams DH, Hubscher S, Hirano H, Siebenlist U & Shaw S (1993) Nature 361: 79–82
28. Günthert U, Hofmann M, Rudy W, Reber S, Zöller M, Haussmann I, Matzku S, Wenzel A, Ponta H & Herrlich P (1991) Cell 65: 13–24
29. Edelman GM & Crossin KL (1991) Annu. Rev. Biochem. 60: 155–190
30. Takeichi M (1991) Science 251: 1451–1455
31. Kemler R (1992) Seminars in Cell Biol. 3: 149–155
32. Gumbiner B, Stevenson B & Grimaldi A (1988) J. Cell Biol. 107: 1575–1587
33. Shore EM & Nelson WJ (1991) J. Biol. Chem. 266: 19672–19680
34. Magee AI & Buxton RS (1991) Current Opinion in Cell Biol. 3: 854–861
35. Rouiller DG, Cirulli V & Halban PA (1991) Dev. Biol. 148: 233–242
36. Ozawa M & Kemler R (1992) J. Cell Biol. 116: 989–996
37. Stappert J & Kemler R (1993) Curr. Opinion Neurobiol. 3: 60–66
38. Herrenknecht K, Ozawa M, Eckerskorn C, Lottspeich F, Lenter M & Kemler R (1991) Proc. Natl. Acad. Sci. USA 88: 9156–9160
39. McCrea PD & Gumbiner BM (1991) J. Biol. Chem. 266: 4514–4520
40. Butz S, Stappert J, Weissig H & Kemler R (1992) Science 257: 1142–1144
41. Takeichi M (1977) J. Cell Biol. 75: 464–474
42. Kadmon G, Korvitz A, Altevogt P & Schachner M (1990) J. Cell Biol. 110: 193–208
43. Shimoyama Y, Nagafuchi A, Fujita S, Gotoh M, Takeichi M, Tsukita S & Hirohashi S (1992) Cancer Res. 52: 5770–5774
44. Matsuyoshi N, Hamaguchi M, Tanigushi S, Nagafuchi A, Tsukita S & Takeichi M (1992) J. Cell Biol. 118: 703–714
45. Hamaguchi M, Matsuyoshi N, Ohnishi Y, Gotoh B, Takeichi M & Nagai Y (1993) The EMBO J. 12: 307–314
46. Luna EJ & Hitt AL (1992) Science 258: 955–964
47. Behrens J, Mareel MM, Van Roy FM & Birchmeier W (1989) J. Cell Biol. 108: 2435–2447
48. Vleminckx K, Vakaet L Jr, Mareel M, Fiers W & Van Roy F (1991) Cell 66: 107–119
49. Mareel M, Vleminckx K, Vermeulen S, Bracke M & Van Roy F (1992) Bull. Cancer 79: 347–355
50. Vakaet L Jr, Vleminckx K, Van Roy F & Mareel M (1991) Invasion and Metastasis 11: 249–260
51. Pignatelli M, Liu D, Nasim MM, Stamp GWH, Hirano S & Takeichi M (1992) Br. J. Cancer 66: 629–634

52. Tang A, Amagai M, Granger LG, Stanley JR & Udey MC (1993) Nature 361: 82–85
53. Mareel M, Van Roy F, De Baetselier P & Vakaet L. In: Hodges GM & Rowlatt C (Eds) Developmental biology and cancer. The Telford Press, New Jersey, USA (in press)
54. Hyafil F, Babinet C & Jacob F (1981) Cell 26: 447–454
55. Mahoney PA, Weber U, Onofrechuk P, Blessmann H, Bryant PJ & Goodman CS (1991) Cell 67: 853–868
56. Fearon ER & Vogelstein B (1990) Cell 61: 759–767
57. Kikuchi-Yanoshita R, Konishi M, Fukumari H, Tanaka K & Miyaki M (1992) Cancer Res. 52: 3801–3803
58. Hedrick L, Cho KR & Vogelstein B (1993) Trends Cell Biol. 3: 36–39
59. Navarro P, Gomez M, Pizarro A, Gamallo C, Quintanilla M & Cano A (1991) J. Cell Biol. 115: 517–533
60. Redies C, Inuzuka H & Takeichi M (1992) J. Neuroscience 12: 3525–3534
61. Sefton M, Johnson MH & Clayton L (1992) Development 115: 313–318
62. Hyafil F, Morello D, Babinet C & Jacob F (1980) Cell 21: 927–934
63. Peyriéras N, Hyafil F, Louvard D, Ploegh HL & Jacob F (1983) Proc. Natl. Acad. Sci. USA 80: 6274–6277
64. Edelman GM, Gallin WJ, Delouvee A, Cunningham BA & Thiery JP (1983) Proc. Natl. Acad. Sci. USA 80: 4384–4388
65. Vakaet L (1970) Arch. Biol. (Liège) 81: 387–426
66. Vestweber D, Kemler R & Ekblom P (1985) Dev. Biol. 112: 213–221
67. Fleming TP, Garrod DR & Elsmore AJ (1991) Development 112: 527–539
68. Logan SK, Fisher SJ & Damsky CH (1992) Cancer Res. 52: 6001–6009
69. Coutifaris C, Kao L-C, Sehdev HM, Chin U, Babalola GO, Blaschuk OW & Strauss JF III (1991) Development 113: 767–777
70. Shimamura K, Takahashi T & Takeichi M (1992) Dev. Biol. 152: 242–254
71. Mareel M, Bracke M, Van Roy F & Vakaet L (1993) Int. J. Dev. Biol. 37 (in press)
72. Ruggeri B, Caamano J, Slaga TJ, Conti CJ, Nelson WJ & Klein-Szanto AJP (1992) Am. J. Pathol. 140: 1179–1185
73. Eidelman S, Damsky CH, Wheelock MJ & Damjanov I (1989) Am. J. Pathol. 135: 101–110
74. Oka H, Shiozaki H, Kobayashi K, Tahara H, Tamura S, Miyata M, Doki Y, Iihara K, Matsuyoshi N, Hirano S, Takeichi M & Mori T (1992) Virchows Arch. A Pathol. Anat. 421: 149–156
75. Van Der Wurff AAM, Ten Kate J, Van Der Linden EPM, Dinjens WNM, Arends J-W & Bosman FT (1992) J. Pathol. 168: 287–291
76. Behrens J, Frixen U, Schipper J, Weidner M & Birchmeier W (1992) Seminars in Cell Biology 3: 169–178
77. Furukawa F, Miyachi Y, Wakai Y & Imamura S (1991) Int. J. Dermatol. 30: 599–600
78. Rocco MV, Neilson EG, Hoyer JR & Ziyadeh FH (1992) Am. J. Physiol. 262: F679–F686
79. Takahashi M, Fujimoto T, Honda Y & Ogawa K (1992) Invest. Ophthalmol. Visual Sci. 33: 280–285
80. McNeill H, Ozawa M, Kemler R & Nelson WJ (1990) Cell 62: 309–316
81. Frixen UH, Behrens J, Sachs M, Eberle G, Voss B, Warda A, Löchner D & Birchmeier W (1991) J. Cell Biol. 113: 173–185
82. Chen W & Öbrink B (1991) J. Cell Biol. 114: 319–327
83. Mareel M, Vleminckx K, Vermeulen S, Gao Y, Vakaet L Jr, Bracke M & Van Roy F (1992) In: Graumann W & Drukker J (Eds) Progressin histo- and cytochemistry vol 26 (pp 95–106) Fisher Verlag, Stuttgart/Jena/New York
84. Mareel MM & Van Roy FM (1986) Anticancer Res. 6: 419–436
85. Mareel MM, Behrens J, Birchmeier W, De Bruyne GK, Vleminckx K, Hoogewijs A, Fiers WC & Van Roy FM (1991) Int. J. Cancer 47: 922–928
86. Gao Y, Vleminckx K, Van Roy F & Mareel M (1992) Clin. Exp. Metastasis 10 (Suppl. 1): 11
87. Gabbert HE, Meier S, Gerharz CD & Hommel G (1992) Int. J. Cancer 50: 202–207
88. Ozawa M, Engel J & Kemler R (1990) Cell 63: 1033–1038
89. Carter BS, Ewing CM, Ward WS, Treiger BF, Aalders TW, Schalken JA, Epstein JI & Isaacs WB (1990) Proc. Natl. Acad. Sci. USA 87: 8751–8755
90. Thomas GA & Raffel C (1991) Cancer Res. 51: 639–643
91. Sato T, Tanigami A, Yamakawa K, Akyiama F, Kasumi F, Sakamoto G & Nakamura Y (1990) Cancer Res. 50: 7184–7189
92. Tsuda H, Zhang W, Shimosato Y, Yokota J, Terada M, Sugimura T, Miyamura T & Hirohashi S (1990) Proc. Natl. Acad. Sci. USA 87: 6791–6794
93. Huff V, Reeve AE, Leppert M, Strong LC, Douglass EC, Geiser CF, Li FP, Meadows A, Callen DF, Lenoir G & Saunders GF (1992) Cancer Res. 52: 6117–6120
94. Maw MA, Grundy PE, Millow LJ, Eccles MR, Dunn RS, Smith PJ, Feinberg AP, Law DJ, Paterson MC, Telzerow PE, Callen DF, Thompson AD, Richards RI & Reeve AE (1992) Cancer Res. 52: 3094–3098
95. Weng DE & Littlefield JW (1991) Somat. Cell Mol. Genet. 17: 617–619
96. Bussemakers MJG, Van Moorselaar RJA, Giroldi LA, Ichikawa T, Isaacs JT, Takeichi M, Debruyne FMJ & Schalken JA (1992) Cancer Res. 52: 2916–2922
97. Wollner DA, Krzeminski KA & Nelson WJ (1992) J. Cell Biol. 116: 889–899
98. Roark EF, Paradies NE, Lagunowich LA & Grunwald GB (1992) Development 114: 973–984
99. Nagafuchi A & Takeichi M (1990) Cell Regul. 1: 37–44
100. Kintner C (1992) Cell 68: 225–236
101. Hatta K & Takeichi M (1986) Nature 320: 447–449
102. Choi Y-S & Gumbiner B (1989) J. Cell Biol. 108: 2449–2458
103. Choi Y-S, Sehgal R, McCrea P & Gumbiner B (1990) J. Cell Biol. 110: 1575–1582
104. Angres B, Muller AHJ, Kellermann J & Hausen P (1991) Development 111: 829–844

105. Ginsberg D, DeSimone D & Geiger B (1991) Development 111: 315–325
106. Levi G, Gumbiner BM & Thiery JP (1991) Development 111: 159–169
107. Detrick RJ, Dickey D & Kintner CR (1990) Neuron 4: 493–506
108. Hirano S, Kimoto N, Shimoyama Y, Hirohashi S & Takeichi M (1992) Cell 70: 293–301
109. Volberg T, Geiger B, Dror R & Zick Y (1991) Cell Regul. 2: 105–120
110. Behrens J, Vakaet L, Friis R, Winterhager E, Van Roy F, Mareel MM & Birchmeier W (1993) J. Cell Biol. 120: 757–766
111. Reynolds AB, Herbert L, Cleveland JL, Berg ST & Gaut JR (1992) Oncogene 7: 2439–2445
112. Guan J-L & Shalloway D (1992) Nature 358: 690–692
113. Jouanneau J, Tucker GC, Boyer B, Vallés AM, Thiery JP (1991) Cancer Cells 3: 525–529
114. Prozialeck WC & Niewenhuis RJ (1991) Biochem. Biophys. Res. Commun. 181: 1118–1124
115. Cyr DG, Blaschuk OW & Robaire B (1992) Endocrinology 131: 139–145
116. Bracke ME, Van Larebeke NA, Vyncke BM & Mareel MM (1991) Br. J. Cancer 63: 867–872
117. Shao ZM, Sheikh MS, Ordonez JV, Feng P, Kute T, Chen JC, Aisner S, Schnaper L, LeRoith D, Roberts CT Jr & Fontana J (1992) Cancer Res. 52: 5100–5103
118. Remacle-Bonnet M, Garrouste F, El Atiq F, Roccabianca M, Marvaldi J & Pommier G (1992) Int. J. Cancer 52: 910–917
119. O'Farrell PH (1975) J. Biol. Chem. 250: 4007–4021
120. Heukeshoven J & Dernick R (1985) Electrophoresis 6: 103–112
121. Shiozaki H, Tahara H, Oka H, Miyata M, Kobayashi K, Tamura S, Iihara K, Doki Y, Hirano S, Takeichi M & Mori T (1991) Am. J. Pathol. 139: 17–23
122. Gamallo C, Palacios J, Suarez A, Pizarro A, Navarro P, Quintanilla M & Cano A. Am. J. Pathol. (in press)
123. Shimoyama Y, Hirohashi S, Hirano S, Nogushi M, Shimosato Y, Takeichi M & Abe O (1989) Cancer Res. 49: 2128–2133
124. Dorudi S, Sheffield JP, Poulsom R, Northover JMA & Hart I (1992) Clin. Exp. Metastasis 10 (Suppl. 1): 39
125. Van Aken J, Cuvelier C, Gao Y, De Wever N, Roels H & Mareel M (1991) Pathol. Res. Pract. 187: 672
126. Umbas R, Schalken JA, Aalders TW, Carter BS, Karthaus HFM, Schaafsma HE, Debruyne FMJ & Isaacs WB (1992) Cancer Res. 52: 5104–5109
127. Schipper JH, Frixen UH, Behrens J, Unger A, Jahnke K & Birchmeier W (1991) Cancer Res. 51: 6328–6337
128. Shimoyama Y & Hirohashi S (1991) Cancer Res. 51: 2185–2192
129. Inoue M, Ogawa H, Miyata M, Shiozaki H & Tanizawa O (1992) Am. J. Clin. Pathol. 98: 76–80
130. Tohma Y, Yamashima T & Yamashita J (1992) Cancer Res. 52: 1981–1987
131. Czech W, Krutmann J, Herrenknecht K, Kapp A & Schöpf E (1990) J. Invest. Dermatol. 94: 517