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Cell adhesion in cancer

Adhérence cellulaire et cancer

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Presented by Guy Laval

Abstract

Cell adhesion is a key physiological event tightly coupled to other major cellular processes coordinating morphogenesis and histogenesis. Cell-to-cell and cell-to-extracellular matrix adhesion regulates the social behavior of cells in developing embryos and in the adult. These two adhesion systems also play a critical role in pathogenesis. In vertebrates, more than 3% of genes are thought to encode adhesion molecules. The largest cell adhesion molecule superfamily is that related to N-CAM, the members of which characteristically contain immunoglobulin domains. Cadherins, which also possess Ig domains, constitute another important superfamily with different properties. Integrins are major receptors for many extracellular matrix components. This review describes the structure and function of these adhesion systems and their impact in cancer invasion and metastasis. ***To cite this article: J.P. Thiery, C. R. Physique 4 (2003).***

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Résumé

L'adhérence cellulaire est un mécanisme physiologique contrôlant la morphogenèse l'histogenèse et l'intégrité des tissus adultes par son couplage étroit avec les processus de migration, de prolifération, de différenciation et de mort cellulaire. Cette revue rappelle la structure et la fonction des molécules adhésives appartenant à la super famille des immunoglobulines, des cadhérines et des intégrines qui assurent les interactions entre cellules et entre cellules et matrice extracellulaire. Le rôle de ces molécules adhésives est décrit dans les mécanismes de progression des cancers. ***Pour citer cet article : J.P. Thiery, C. R. Physique 4 (2003).***

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Mots-clés : Adhérence cellulaire ; CAM-Ig ; Cadhérines ; Intégrines ; Carcinomes ; Progression

1. Introduction

Cell adhesion is a key physiological event tightly coupled to four other major cellular processes: proliferation, migration, differentiation and death. Any change in cell adhesion has major consequences for the behavior of cells, and may even result in the induction of cell death. Cell adhesion studies have now reached a 'golden age' [1], with cell adhesion molecules thought likely to account for more than 3% of the proteins encoded by the human genome. Many different signal transduction pathways have been discovered and partly elucidated and cell adhesion molecules are increasingly being implicated in various diseases. Research into cell adhesion focuses on two areas: intercellular adhesion and cell-to-substratum adhesion. However, certain

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adhesion molecules have been implicated in both types of adhesion and there is increasing evidence for cross-talk between intercellular and cell-to-substratum adhesive events. In this review, I will present a very brief historical overview of this field and will then describe major aspects of the structure-function relationships of various adhesion molecule superfamilies. This will lead on to a discussion of the potential involvement of adhesion molecules in the emergence and progression of carcinomas.

2. Historical background

Developmental biologists have been interested in cell-cell adhesion processes for more than a century. Jean Baptiste Robinet (1735–1820) first described the strength of spermatocyte adhesion in a book entitled '*De la Nature*', Amsterdam, E. van Harrevelt (1761). In 1925, Galstoff [2] showed that, following reassociation, sponge cells of two different genera, identified on the basis of their different colors, gradually sorted themselves into two distinct aggregates. Similar studies were later carried out with amphibian embryonic cells of epidermal and neural origin, to assess the degree of cell recognition [3]. Various hypotheses have been put forward to account for the ability of different cell types in mosaic aggregates to sort themselves out and to reestablish tissue-like structures. In his differential adhesion hypothesis, Steinberg [4] suggested that the sorting process is driven thermodynamically, moving towards maximum thermodynamic stability. More recent studies have emphasized the role of interfacial surface tension: the tissues with the highest surface tension are always located within the histiotypic aggregate [5]. However, neither of these approaches resulted in the identification of molecular structures potentially involved in the recognition and sorting processes. The first attempts to identify the molecular components involved in adhesion and recognition processes were made in the late 1960's, with chick embryonic neural cells. These studies led to the discovery of cognins [6], the functions of which were never determined. In 1963, Robert Sperry [7] proposed that an extremely large repertoire of molecules in the nervous system constitutes a molecular code specifying neural networks, particularly for synaptic contacts (of the order of 10^{15} molecules in the human brain), complicating the task of researchers in this field. However, the very existence of specific adhesion/recognition molecules was questioned by a number of investigators who suggested that cell recognition was mediated solely by nonspecific electrostatic and Van der Waals interactions [8].

In the early 1970s, several laboratories adopted new approaches to address the crucial question of whether histogenesis and organogenesis involved bona fide cell adhesion molecules. Scientists in the laboratory of Gerald Edelman were the first to attempt to identify a cell recognition molecule in the nervous system. A cell surface molecule approximately 140 kDa in size was found to mediate the adhesion of neural cells in chick embryos; this molecule was therefore called N-CAM (neural cell adhesion molecule) [9]. In François Jacob's laboratory, Hyafil and coworkers identified a molecule that they called uvomorulin; this molecule was initially identified as potentially involved in compaction of the 8-cell mouse morula [10]. An antibody that disturbed epithelial adhesion was identified in the laboratory of Walter Birchmeier [11]. The calcium-dependent adhesion mechanism identified by Masatoshi Takeichi [12] was later shown by the teams of Takeichi and Rolf Kemler [13] to be related to uvomorulin, which is now called E-cadherin (epithelial calcium-dependent cell adhesion molecule). At around the same time, Gerald Edelman and his team identified L-CAM, a calcium-dependent cell adhesion molecule expressed in liver of chick embryos [14]. Further studies revealed that L-CAM was an ortholog of mouse E-cadherin [15,16]. In 1983, the patterns of expression of N-CAM and L-CAM during embryogenesis were established. These patterns suggested that these two molecules were involved in the formation of different tissues in the developing embryonic primordia [1]. Several other molecules belonging this nascent family of cadherins have been cloned [17]. One major surprise was the identification of five potential immunoglobulin domains in the extracellular region of N-CAM, on the basis of the primary sequence, established in 1986 [18]. A large number of molecules related to N-CAM were subsequently identified. These molecules were predominantly expressed in the nervous system but were also present in other tissues and in the immune system [19]. The discovery of an ortholog, Fasciclin2, in *Drosophila* [20] raised the intriguing possibility that members of the N-CAM family evolved earlier than immunoglobulins and that Ig-domains were originally selected as primary recognition systems, well before developing their antigen recognition function in the immune system [21]. Cadherins were also recently shown to contain Ig domains, although in this case the discovery was based not on primary structure, but on studies of the three-dimensional structure of the amino-terminal fragments of these molecules [22,23].

Other cell surface molecules not belonging to the N-CAM and cadherin superfamilies were identified as regulators of intercellular adhesion in the 1980s and 1990s. These molecules include selectins and their cognate receptors, which are involved in controlling the arrest and subsequent extravasation of monocytes and lymphocytes in blood and lymph vessels [24,25]. The putative intercellular adhesion molecule EpCAM, discovered in the 1970s as a carcinoma antigen, is also ubiquitously produced by normal and malignant epithelial cells, but its adhesive function is still a matter of debate [26]. Other adhesion molecules, such as CAM105, are present in various cell types, including a number of epithelial cell types [27]. It should be noted that some families of surface receptors and ligands induce repulsion rather than stable adhesion. These families include the ephrins, the semaphorins and their cognate receptors, which are produced in many cell types during embryogenesis and control tissue segregation and axon guidance by inducing repulsion upon adhesive contact [28,29]. The surface receptor Notch was discovered

by genetic screening in *Drosophila*. It was initially identified as a gene encoding a surface protein controlling cell fate, but it may also act as a cell adhesion molecule in many tissues. The constitutive activation of this gene may lead to oncogenic transformation in cooperation with ras [30,31].

Research into the adhesion of cells to the substratum has also gone through a long lag phase. It was not until the 1950s that it was recognized that cell attachment, spreading and locomotion *in vitro* required specific interactions with the substratum [32]. Electron microscopy studies showed that, *in vivo*, this substratum is composed of fibrillar structures and amorphous material. Collagen (now called type I collagen) was one of the first components of the extracellular matrix to be discovered. Other macromolecules, such as hyaluronic acid, heparan sulfate and chondroitin sulfate proteoglycans, have also been identified as integral components of the extracellular matrix [33]. In the mid-1970s, a new protein that adhered strongly to the substratum was discovered in a number of laboratories, resulting in its being baptized with as many as 17 different names. This protein is now called fibronectin. It was studied extensively in the 1980's, but the three-dimensional structures of its three characteristic protein domains – FN1, FN2 and FN3 – were established only recently [34–36].

Laminin, another component of the extracellular matrix, was identified in 1979 [37]. This macromolecule was rapidly identified as a key component of the basement membranes lining epithelial tissues. Studies of extracellular matrix molecules progressed rapidly with the discovery of more than 18 collagens and 12 laminins, and of many other extracellular matrix components including tenascins, thrombospondins, fibulins and SPARC (http://web.mit.edu.ccrhq/hyneslab).

The surface receptors for the various extracellular matrix components remained elusive until the mid-1980s, when the first integrin receptor was discovered (references cited in [38]). These heterodimeric receptors now form a family with 24 known members. Other receptors have been identified, including syndecans. These proteoglycans were originally identified on the basis of their ability to bind collagen 1 [39,40]. Other proteoglycan receptors include CD44, which specifically recognizes hyaluronan [41] and dystroglycans, which bind laminins and other extracellular matrix components [42,43]. Cell adhesion has thus become an extremely complex field, not only because a large number of families of molecules are involved, but also because the mechanisms regulating this process are diverse. It is particularly difficult, both theoretically and technically, to quantify physical parameters describing the adhesive status of cells in contact with substrata or interacting with other cells [44,45]. In addition, state-of-the art signal transduction analysis has not yet reached a level of definition sufficiently high for signals resulting from adhesive interactions to be followed in real time. Indeed, no algorithm currently exists for predicting the adhesive status of a cell at any given moment of its life in the embryo or adult.

Fig. 1 presents a highly simplified representation of the repertoires of various adhesive receptors. Note considerable variation in motives and sizes of the molecules.

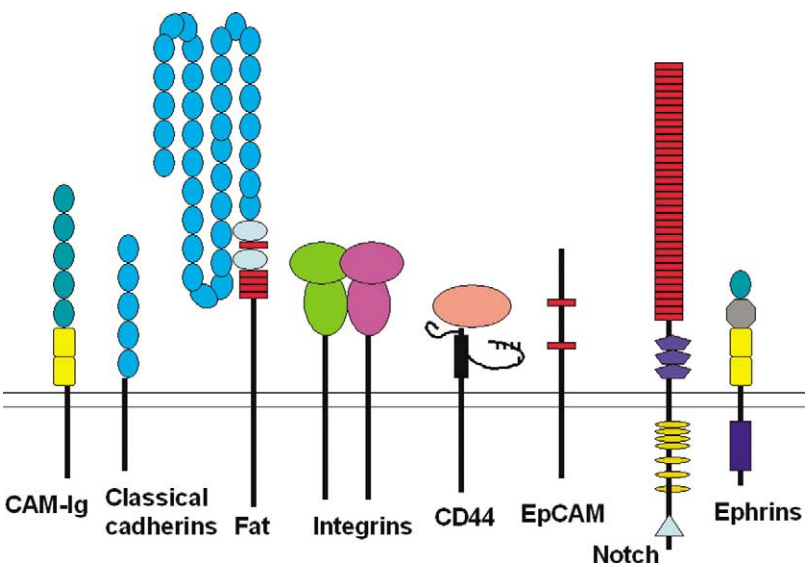

Fig. 1. A highly simplified representation of the repertoires of various adhesive receptors. Cyan and blue ovals: Ig domains; yellow rectangle: FN3 motifs, red rectangles: EGF-like motif; black rectangle: variable domain; light blue ovals: laminin-A G repeat; pink oval: carbohydrate binding site blue pentagon: notch-lin 12 repeats; yellow ovals: ankyrin repeats; light blue triangle: pest sequence blue; grey hexagon: cystein-rich region; blue rectangle: tyrosine kinase domain.

3. The Ig superfamily of cell adhesion molecules

The discovery of the five immunoglobulin domains in the extracellular part of N-CAM was rapidly followed by the identification of other adhesion molecules with similar patterns of protein folding. An Ig domain is formed by a tightly packed barrel of seven or nine antiparallel beta strands arranged in two layers. This structure is stabilized by a disulfide bond. The Ig superfamily was first recognized by Williams and coworkers [46], and has since expanded to 765 members in humans [47,48]. Many of these molecules were identified by screening of the human genome database. This is clearly one of the largest protein superfamilies. The three-dimensional structure of several Ig domains has been established; each Ig domain is about $25 \times 30 \times 40$ Å in size and homophilic interactions between Ig domains may involve an area of approximately 800 $Å^2$. Ig domain structures display considerable diversity, as described in a previous review [49]. The number of Ig domains in the extracellular region of the proteins of this superfamily varies from 1 to 17. Splicing mechanisms have been shown to generate multiple transcripts in a number of genes encoding Ig-CAM. The most impressive splicing process is probably that of DS-CAM, a *Drosophila* Ig-CAM, that may theoretically generate as many as 38 000 different proteins [50]. In addition to bona fide Ig domains, a number of structurally related motifs called fibronectin III (FN-III) type domains are present in the extracellular regions of many Ig superfamily members [51]. The classification of Ig superfamily members is not an easy task. Numerous growth factors and cytokine receptors such as PDGF, FGF, C-kit, CSF-1, IL-1, and IL-6, are included in this superfamily. Proteins associated with immune recognition, such as MHC class 1 and class 2, TCR and associated proteins contain Ig domains. The families of adhesion molecules have been also grouped into subcategories on the basis of structure, distribution and function. For instance, N-CAM, and the recently discovered closely related molecules O-CAM and P-CAM [52], can be classified with adhesion proteins involved in myelination such as MAG. L1/Ng-CAM can be grouped with Nr-CAM/Bravo and Neurofascin. Another group includes contactin/F11, TAG1 and axonin. The roles of all these molecules in the nervous system have been partly elucidated. Other groups of molecules including I-CAM and V-CAM have been implicated in endothelial cell recognition by blood cells. Yet another family brings together CEA and pregnancy-specific glycoproteins (PSG) such as CAM105, which is also known as C-CAM. A number of functions have been attributed to the members of this family. In most cases, these adhesion molecules act by means of a calcium-independent mechanism, but there are notable exceptions, such as PE-CAM and C-CAM. These adhesion molecules exert their effects via a homophilic or a heterophilic mechanism. For instance N-CAM, binds to itself in cell-cell contact but may also bind heterophilically to axonin/TAG-1, neurocan and the phosphacan PTB β/ζ phosphatase surface receptor. Some CAM-Ig, such as I-CAM and V-CAM, bind to integrins, a very different set of adhesion receptors (see section on integrins) [19].

In the last few years, it has become apparent that CAM-Ig can interact in *cis* with specific partners. N-CAM and DCC display self-association. DCC undergoes multimerization following binding to netrin, an extracellular matrix (ECM) guidance molecule for commissural neurons. DCC also binds to robot, a receptor for the central nervous system midline repellent, slit. Most, but not all members of the Ig superfamily have a transmembrane domain and a cytoplasmic domain, facilitating binding to adaptor proteins and to other signaling molecules. In particular, JAM, nectins and neurofascin bind afadins I and S via their PDZ domains. I-Afadin also possesses an actin-binding site, responsible for connecting JAM or nectins to actin microfilaments. I-Afadin is therefore a critical element in the organization of junctional complexes [53] (see section on epithelial junctions).

4. Cadherins

4.1. Classical cadherins

The first cadherins to be identified were named according to their tissue of origin [54]. E-cadherin is present primarily in epithelial tissues. However, N-cadherin, given this name because it was initially found in the nervous system, is also found outside this system. Similarly, P-cadherin is present in many tissues other than the placenta. These cadherins are closely related proteins placing them in the family of classical cadherin type 1. They contain 5 extracellular repeats, each approximately 110 amino acids in length. These repeats numbered EC1 to EC5, with EC1 closest to the extracellular matrix and EC5 closest to the plasma membrane, are now known to fold into Ig domains.

Other closely related molecules that diverge somewhat in their primary sequences have been clustered into the classical cadherin type 2 group [55]. These cadherins are designated by numbers: for example, cadherin 5 is also known as vascular cadherin. Mammals may have more than 20 classical cadherins [56]. However, this nomenclature is not rigorous because cadherin 13, also known as T-cadherin, lacks the transmembrane and cytoplasmic domains and remains anchored to the plasma membrane via a glycosyl phosphatidyl inositol linker moiety. A number of classical cadherins are encoded by genes clustered at 16q22, a locus often involved in loss of heterozygozity in cancer [57].

Classical cadherins display almost exclusively homophilic binding. Early studies on the structure/function relationship clearly demonstrated the importance of the amino-terminal EC1 domain. The first three-dimensional structure to be published

for a molecule of this group was the amino-terminal domain of N-cadherin, which was obtained in 1995 [22]. This study revolutionized thinking in this field as it demonstrated, for the first time, the presence of an Ig fold in the extracellular repeats of a cadherin molecule. Analysis of the structures of *cis* and *trans* dimers suggested a linear zipper model for adhesion. In this model, cadherin *cis* dimers at the plasma membrane interact in *trans* with *cis* dimers from the other cell surface in contact. This interaction would involve direct contact between the amino-terminal EC1 domains. Although highly attractive, this model was later revised because the structure considered was derived from a domain lacking calcium binding sites. Structures established for EC1-EC2 domains provided no evidence for *cis* or *trans* dimerization of the zipper type [58–60]. The most recent structure to be published was established from crystals of the complete extracellular region of a classical cadherin [23]. Each cadherin molecule in the cluster is curved such that EC5, the domain proximal to the plasma membrane, is perpendicular to EC1, the amino-terminal domain. *Cis* multimers involve interactions between consecutive EC1 and EC2 domains. A symmetric reciprocal exchange of amino-terminal β strands between the terminal domains of molecules interacting in *trans* has been demonstrated, together with the symmetric insertion of tryptophan 2 into the apposed domain. The intercellular space between cells in contact may be significantly smaller than that estimated in the zipper model, possibly corresponding to the distance of 25 nanometers between cells in contact observed by electron microscopy. Such studies by crystallography and other methods, including the negative staining and electron microscopy of cadherins, may not yet have revealed the exact structure of the molecule. It is also unclear whether the tryptophan in position 2, which is essential for adhesion, is inserted into its own domain or adjacent domains in *cis* or in *trans*. It is not yet clear what determines the specificity of the interaction for each classical cadherin, because the sequences involved in homophilic interactions are strongly conserved between the various members of the family. Cadherins may also present different conformations at the cell surface, which may be modified upon contact in *trans*. In any case, multimer formation is essential to the avidity of the interaction. There is also evidence that *trans* interaction may involve more than just the EC1 domain. Leckband and coworkers [61] put forward a modified zipper model in which the apposed extracellular domains interact along their entire length. The k_{off} of E-cadherin EC1-EC2 fragments was recently established and showed that the interaction lasted 2 seconds [62]. A similarly brief interaction is also observed for selectins. Therefore, the strong E-cadherin-mediated interactions observed in cells, of the order of several hundred nanonewtons (Dufour et al., unpublished) are very likely to depend on cooperative mechanisms within cadherin clusters.

The cytoplasmic region of classical cadherins is approximately 170 amino acids long and is of critical importance in the mechanical and chemical transduction of adhesive mechanisms. Several proteins have been shown to form a complex with the cytoplasmic region [63]. The proteins known as catenins (taken from the word for chain in Latin) are now very well characterized. β-catenin and γ-catenin (plakoglobin) interact directly with the cytoplasmic region via their central domain, which consists of a series of Armadillo repeats. These repeats of a 42-amino acid sequence that has been identified in numerous proteins form an extended polypeptide chain with alternating positive and negative areas [64]. The amino- and carboxy-terminal domains not engaged in interaction with catenins have other critical functions. The amino-terminal domains of β-catenin and plakoglobin bind α-catenin, a protein structurally similar to vinculin. These two proteins can interact directly with α-actinin, thereby forming a connection between actin microfilaments and cadherins. P120 is another member of the catenin Armadillo family; it binds to the cytoplasmic region of cadherins at a site more proximal to the transmembrane domain. However, P120 is not connected to the actin cytoskeleton. Instead, it fulfills other functions in signal transduction by interacting with the small GTPase RhoA. There is evidence to suggest that P120 is involved in cadherin clustering, strengthening cell adhesion. However, P120 may also increase cell motility by activating CDC42 and Rac GTPases [65].

IQGAP-1 also regulates cadherin-mediated adhesion. In conditions of low intracellular calcium concentration, IQGAP-1 interacts with activated CDC42 and Rac at the cell surface and connects actin filaments to calmodulin. These complexes stabilize cadherin-catenin-cytoskeleton complexes. In conditions of high intracellular calcium concentration, the calmodulin IQGAP-1 complex dissociates from the small GTPases and IQGAP-1 displaces α-catenin from β-catenin, thereby disrupting the connection to the actin microfilament, decreasing adhesion [57].

Perhaps one of the most important discoveries in this field is the observation that, under certain circumstances, β-catenin is also found in the nucleus. β-catenin, which has importin-like sequences may pass through the nuclear pores alone or in complexes with the LEF-1/TCF transcription factor of the HMG box family [66]. β-catenin therefore acts as a co-transcriptional activator in a large protein complex. Several target genes have been identified, including genes involved in proliferation such as that encoding cyclin D1, and genes involved in invasion, such as MMP7 [67,68]. Studies carried out in Drosophila had already partly elucidated the role of Armadillo, the *Drosophila* β-catenin, in the control of segmental polarity. The Wnt pathway has been shown to control cytoplasmic levels of β-catenin via a transduction pathway involving Dishevelled, an adaptor protein, and Zest white 3/GSK3β, a ubiquitous serine threonine kinase. It was then shown that APC, a very large multifunctional protein involved in familial adenopolyposis – a form of hereditary colon cancer – and in about 70% of sporadic colon cancers, binds to β-catenin [69]. Other studies in *Xenopus laevis* demonstrated that β-catenin plays a key role in controlling the formation of the dorso-ventral axis. Nuclear β-catenin-XTCF-3 transiently activates genes playing a critical role in formation of the Spemann organizer, which is subsequently involved in neural induction [70].

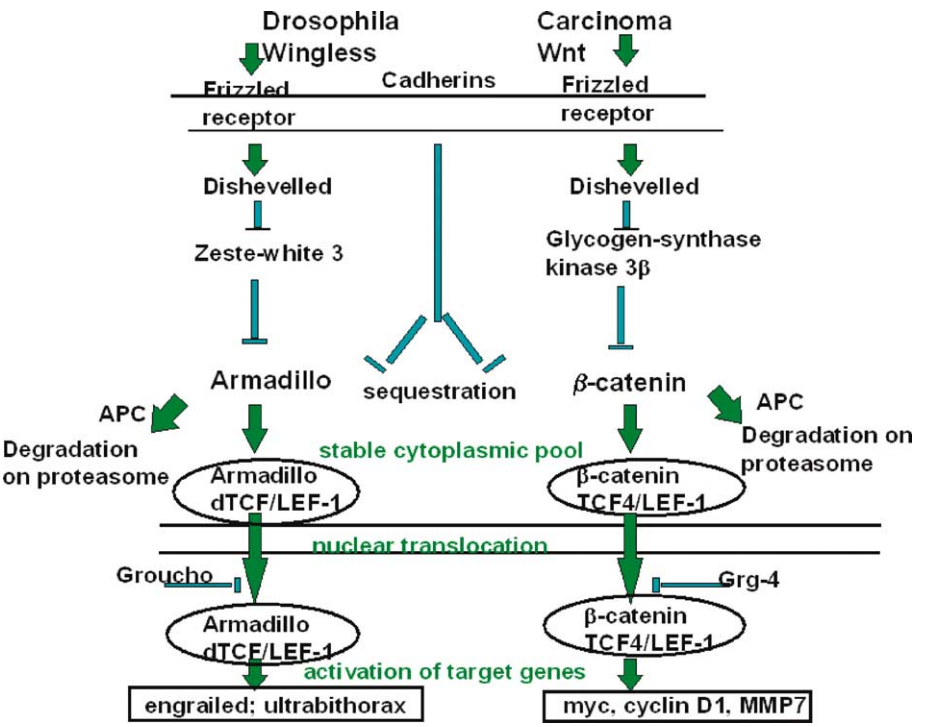

Fig. 2. Similar mechanisms operate in development (*Drosophila*) and cancer, involving the wnt pathway.

A precise model has now emerged for the control of β-catenin pools in the cytoplasm. In the absence of Wnt signaling, β-catenin may be phosphorylated successively by two serine threonine kinases – casein kinase and GSK3β – on several serine residues located in the amino-terminal domain [71]. This phosphorylation occurs in a large complex containing axin/conductin and APC. Once phosphorylated, β-catenin is polyubiquitinated and degraded in the proteasome complex. In the presence of Wnt signaling, Dishevelled is activated by the binding of wnt to its cognate receptor, a seven transmembrane-segment receptor called Frizzled. Dishevelled then inhibits GSK3β, thereby preventing β-catenin from being degraded. β-catenin in the cytoplasm may be sequestered by cadherins or may migrate to the nucleus. The nuclear activity of β-catenin is crucial for inductive events during development, but may have adverse effects in adult cells because β-catenin may act as a potent oncogene [72]. Indeed, mutations in β-catenin that prevent the degradation of this molecule have been described in numerous cancers including hepatocarcinoma, colon carcinoma and melanoma (Van de Wetering, 2002). There is thus a complex relationship between cadherin-mediated adhesion and cancer.

Fig. 2 shows that similar mechanisms operate in development and cancer, involving the wnt pathway, which controls the fate of β-catenin. GSK3-β is inhibited by dishevelled, following wnt binding to frizzled thereby, allowing β-catenin to escape degradation through the APC complex, the ubiquitination machinery and the proteasome. β-catenin in the nucleus serves as a co-transcriptional activator of TCF/LEF displacing groucho, a transcriptional repressor. Target genes of β-catenin can contribute to morphogenesis (polarity in drosophila) and tumor progression in cancer.

4.2. Desmosomal cadherins

Desmocollins and desmogleins, the adhesive components of desmosomes, form a distinct family of 6 proteins encoded by genes clustered on 18q12 [73,74]. Although the extracellular domains of these proteins contain 5 Ig domains similar to those of classical cadherins, their cytoplasmic domains differ significantly. Nonetheless, these cytoplasmic domains possess binding sites for plakoglobin and plakophilins, members of the β-catenin/Armadillo superfamily. Plakoglobin connects the cytoplasmic regions of desmocollin and desmoglein to a large cytoskeletal protein, desmoplakin, which in turn binds to the cytokeratin intermediate filament network. Plakophilin can also bind to desmosomal cadherin, albeit by a different mechanism. It is thought that plakophilin may also recruit desmosomal cadherins at the cell surface and serve other functions in tissue repair, by promoting cell motility in epithelial cells. Plakoglobin and plakophilin can also migrate to the nucleus and serve as transcriptional co-activators. Desmocollins and desmogleins have only recently been demonstrative to have adhesive functions. Heterophilic interactions between desmocollins and desmogleins have been shown to occur in cell-cell adhesion assays, and

specific peptides derived from the putative interaction sites can block adhesion. Phosphorylation of the serine residues of plakoglobin by PKC or of the tyrosine residues of this molecule by a tyrosine kinase surface receptor may also compromise adhesion.

4.3. Protocadherins

Protocadherins [75], which typically have six or seven extracellular cadherin repeats with an Ig fold [76], form the largest family, with more than 60 members. Members of the cadherin-related neuronal receptor subfamily have 6 Ig domains, their intracellular regions differ from that of classical cadherins and they are not thought to interact with catenins. To date, only cytoplasmic tyrosine kinases such as fyn have been shown to bind to the cytoplasmic domain. Within this subfamily, a novel group of molecules, protocadherins, are encoded by genes clustering on chromosome 5q31. They differ in their extracellular regions but share a constant cytoplasmic domain [57]. This new diversity is created by joining one of the many exons, each encoding the entire extracellular domain to the 3 exons encoding the cytoplasmic domain. One subgroup of protocadherins, the μ protocadherins, have only four Ig domains and interact with PDZ-containing cytoplasmic proteins. In total, there may be more than 100 protocadherins, and these molecules are mostly present in the central nervous system. Their possible function in adhesion has yet to be investigated, but they are concentrated with N-cadherin at synaptic contacts.

The Seven transmembrane-span cadherins typically have nine extracellular cadherin repeats. The founding member of this family of cadherins, Flamingo, was originally discovered in *Drosophila*, [77]. Several orthologs of Flamingo have been found in vertebrates. They resemble GPCRs, and may be involved in G protein activation. However, their signaling pathways have yet to be elucidated.

4.4. Fat cadherins

The first member of this family was discovered in *Drosophila*. It contains 34 cadherin repeats, making it the largest cadherin so far described [78]. Dachsous, another member of the *Drosophila* fat cadherin family has 27 repeats. These proteins are probably too large to act as adhesive connectors in cell-cell contact. *Drosophila* mutants with impaired fat cadherin or dachsous have imaginal disc hyperplasia. These proteins may be considered to be tumor suppressors because they control cell proliferation.

5. Integrins

Integrins act principally as adhesive receptors for extracellular matrix components [79]. They assemble as heterodimers of α and β subunits. 18 α subunits and 8 β subunits associate to form 24 different integrins. The β1 chain may associate with as many as 12 α chains. The αv interacts with the β3 subunit whereas the α4 interacts with the β7 subunit. Most integrins recognizes several ECM components, but in rare cases the integrin may principally recognize one molecule. This is the case for α5b1, which recognizes almost exclusively fibronectin. Each ECM component may be recognized by several integrins, demonstrating that most integrins are not strictly specific for one ligand. The structure of the ligand-binding sites of integrins is gradually becoming clearer. The first integrins to be studied recognize an RGD sequence in various ECM proteins. The prototype of this group of integrins is fibronectin. Fibronectin is a multifunctional adhesion protein containing 11 FN3 repeats folded as Ig domains. An additional 4 amino acids, RGDS, inserted into one of the repeats, protrude from the compact Ig domain. These protruding amino acids penetrate into the groove present at the junction between the two integrin subunits. The aspartate residue of the RGDS sequence completes coordination of the calcium ions present in one subunit. Determination of the structure of the I/A domain inserted in half the α subunits led to identification of a metal ion coordination site, which has since been defined as a metal ion-dependent adhesion site (MIDAS). I/A domains may exist in an open or closed configuration. The binding of the ligand induces local conformational changes that propagate along the entire length of the extracellular region of integrins. The open form displays high affinity for ligands whereas the closed form is inactive. In integrins lacking an I/A domain in the α chain, an I/A-like domain is present in the β-chain. This domain interacts with the seven-fold repeat, which adopts a seven-bladed β propeller conformation. This site constitutes the ligand-binding region of ECM components. A detailed model of the three-dimensional structure of the αvb3 integrin is now available for both the inactive and ligand-bound forms [80]. These truly impressive studies have shed light on the way that allosteric changes in integrins rapidly confer specific properties, particularly during platelet aggregation. The cytoplasmic regions of integrins, like those of cadherins, despite comprising just two short polypeptides, interact with a large variety of cytoskeleton components, adaptors and other signaling molecules, including kinases. Recent studies have suggested that the activation signal may be elicited by intracellular events (inside-out signaling) or ligand binding (outside-in signaling). Moreover, the transition from the resting to the activated state depends on the separation of the two cytoplasmic portions of the two subunits. For instance, talin, a large cytoskeletal protein,

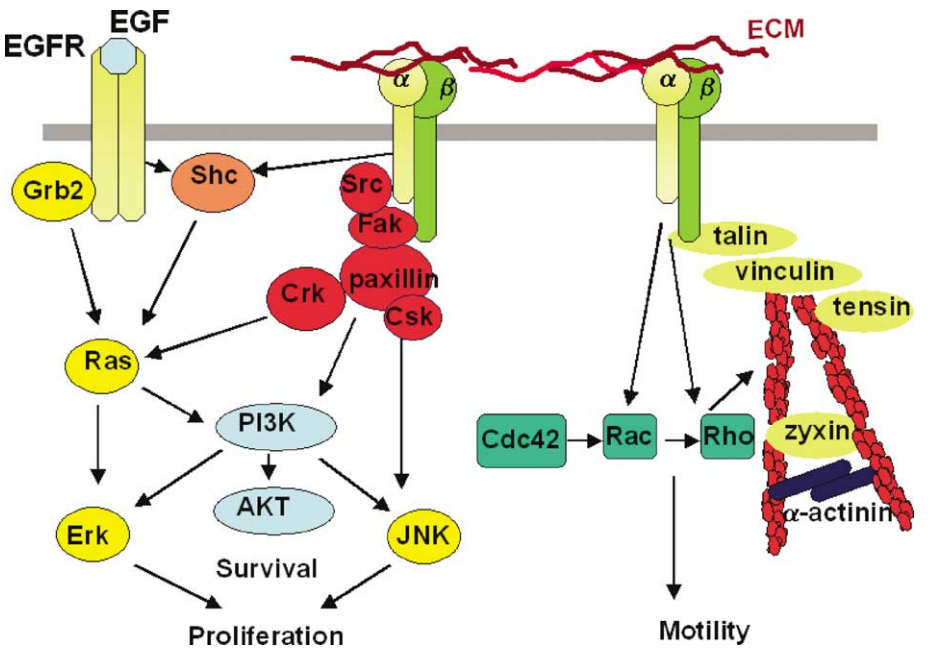

Fig. 3. Thecross-talk between integrin and EGFR-mediated signal transduction pathways and connection to cytoskeletal remodelling. Adaptor proteins containing the SH2 domain such Shc and Crk can connect the two pathways.

can unfold and interact with the tail of the β subunit separating it from its α subunit partner [81,82]. Integrins bind to actin microfilaments, with the exception of the $\alpha 6\beta 4$ integrin, which is found in epithelial cells. This integrin, the β subunit of which has an exceptionally long cytoplasmic tail, interacts with the cytokeratin network via several connectors, in a similar fashion to that observed for desmosomes. Connection to actin microfilaments is mediated by talin and vinculin or by α-actinin, which may also bind the β subunit directly. The FAK tyrosine kinase binds the β subunit directly and acts as a docking site for various substrates, including paxillin or CAS – two closely related multi-adaptor proteins – and the src tyrosine kinase. Multiple complexes, comprising more than 100 proteins, may form at the integrin-cytoplasmic domain interface [83]. These adhesion contacts form at specific sites with the substrates. There are termed focal complex, focal adhesion and fibrillar adhesions to two-dimensional substrates [84]. Each adhesive contact involves the assembly of a different set of cytoplasmic proteins, according to their structure and function in locomotion or in acquisition of the stationary state. Adhesive structures have recently been described in three-dimensional matrices [85]. It is therefore important to consider not only the regulation of affinity by inside-out and outside-in pathways, but also avidity, resulting from the clustering of receptors in the various cell-substrate contact sites.

There are many different integrin signaling pathways, and they are closely connected to the pathways mediated by tyrosine kinase surface receptors [86]. Integrin receptors are therefore major players in four other major processes: cell migration, proliferation, differentiation and death. One important goal of current research is deciphering of the mechanosensor machinery assembled in integrin-mediated adhesions. Any local constraint on cells should immediately be followed by specific local activation of integrin signaling, including reinforcement of the adhesive junction [87]. Finally, cross-talk occurs between the integrin- and cadherin-mediated adhesion processes. This interaction has yet to be explored molecularly but its existence suggests that cells can integrate all adhesive signals and can detect variations in their adhesive phenotypes. Clearly, cancer cells, in which adhesive signal transduction mechanisms have undergone major alterations, must respond differently from normal cells. These complex issues are currently under investigation.

Fig. 3 outlines cross-talk between integrin and EGFR-mediated signal transduction pathways and connection to cytoskeletal remodelling. Adaptor proteins containing the SH2 domain such Shc and Crk can connect the two pathways. Integrin also serve as a platform for the assembly of actin microfilaments.

6. Other adhesive receptors

Syndecans and CD44 are surface proteoglycans that interact with a variety of ECM components. Their cytoplasmic domains interact with cytoskeletal components. These receptors play a major role in specific adhesion in various cell types. Syndecans 1

and 4 seem to act as tumor suppressors in carcinoma, by inhibiting cell migration, whereas syndecan 2 increases the motility and invasiveness of carcinoma cells [88]. CD44 has been thoroughly investigated because the expression of some CD44 isoforms has been shown to be linked to the likelihood of metastasis in carcinoma cells [89]. Ezrin, which binds to the cytoplasmic domain of CD44, regulates cell shape and cell survival, according to its phosphorylation status [90]. CD44 can bind in *cis* to the MT1-MMP metalloprotease, retaining this important enzyme in lamellipodia [91]. Indeed, CD44 acts as a platform for the attachment of various metalloproteases involved in proteolytic degradation of the ECM during migration. For instance, MMP2 may partially cleave partially one chain of laminin 5, generating a motogen. These enzymes may also liberate growth/motility factors trapped in the ECM [92].

7. Epithelial cell junctions, a summary

With the exception of the nervous system, most developing tissues consist of closely interacting epithelial and mesenchymal tissues. In adults, epithelial cells are more prone to cancer than are cells derived from the mesenchyme, and almost 90% of human tumors are carcinomas. If we are to understand the morphological changes and social behavior of carcinoma cells, we need to identify all the potential adhesive sites involved in maintenance of the epithelial cell state. Epithelial cells are highly polarized cells that have three distinct domains at their surface. The apical domain faces the lumen in closed epithelial tissues such as the gut. The lateral domain is mostly involved in junctional complexes, maintaining adhesive contact between epithelial cells, whereas the basal domain interacts with the basement membranes via other specialized junctions.

7.1. Tight junctions

The apical domain is separated from the basolateral domains by tight junctions. The molecular structure of tight junctions has recently been elucidated. Two families of tetraspan proteins, occludins and claudins, have recently been identified at the sites of apposed plasma membranes [93]. Some claudins may create small pores, facilitating fine control of paracellular permeability. JAM adhesion proteins of the CAM-Ig superfamily surround these pores [94]. The cytoplasmic domain of these proteins interacts with zonula adherens proteins, which themselves recruit numerous signaling proteins and actin filaments. One critical issue that remains unresolved concerns whether occludins and claudins act as cell adhesion molecules.

7.2. Adherens junctions

Nectins are three-domain Ig-CAM proteins that seem to be essential for the assembly of adherens junctions. One of their primary functions is the recruitment of E-cadherin. Nectins may form *cis* homodimers that interact in *trans*, but *trans* heterodimers have been detected [95]. The cytoplasmic domains of nectins interact with afadin (see section on CAM-Ig), which may in turn bind actin filaments. Afadin was also recently shown to connect the JAM and E-cadherin protein networks via new linker proteins (Takai, personal communication). Nectins are thus the primary organizers of tight and adherens junctions. Adherens junctions contain large amounts of E-cadherin, which was thought until recently to form homodimers. However, recent crystallographic data suggest that cadherins may form multimers, each cadherin polypeptide interacting in *cis* with the same protein but with a shift in domain-domain interactions such that domain 1 of one cadherin interacts with domain 2 of the neighboring cadherin. Domain 1 is nonetheless involved in the *trans* interaction. The cytoplasmic domain of E-cadherin is associated with catenins, which serve as connectors to the actin cytoskeleton.

7.3. Desmosomes

Desmosomes have long been recognized as adhesive organelle s of epithelial cells. They also consist of cadherins (desmocollins and desmogleins). These cadherins mediate interaction with the cytokeratin intermediate filament network via catenins and desmoplakins [73].

7.4. Gap junctions

Although they are not considered to be adhesive structures, gap junctions facilitate the metabolic and electrical coupling of epithelial cells. Gap junctions are created by the assembly of tetraspan connexin proteins as a hexamer, forming a cylinder with a central channel; two such cylinders from apposed cells then connect together to form a channel between cells [96].

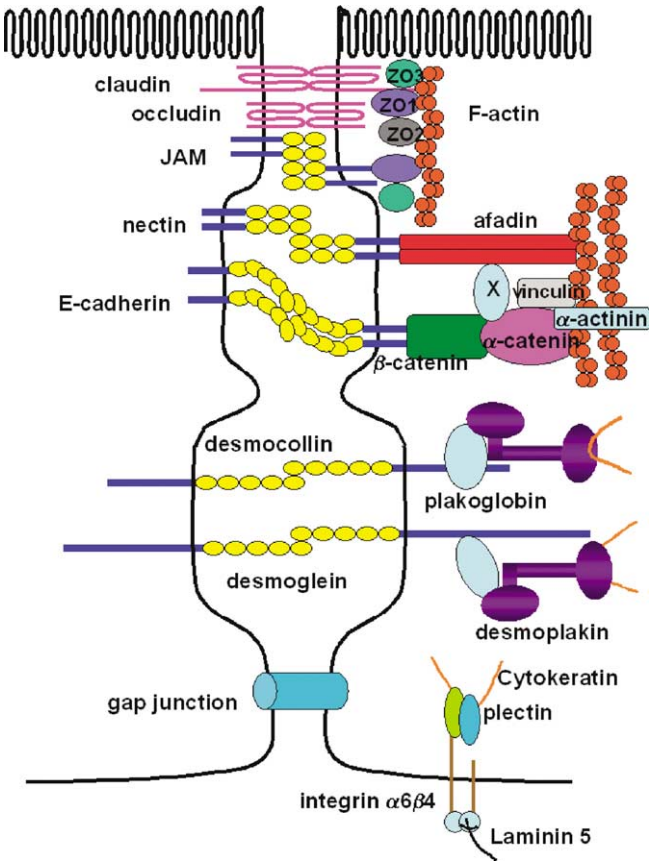

Fig. 4. Schematic diagram of the organization of the various adhesive structures in epithelial cells.

7.5. Hemidesmosomes

Connection to the basal lamina is ensured by various surface receptors, including CD44 and syndecans. Integrins are the most important receptors mediating cell-ECM interactions. Hemidesmosomes essentially consist of the $\alpha 6\beta 4$ integrin, connecting laminin 5 in the basal lamina to a cytokeratin intermediate filament network. This connection is made by means of the remarkably long cytoplasmic domain of $\beta 4$. In addition to the $\alpha 6\beta 4$ adhesive receptor, the transmembrane collagen 17 acts as a coreceptor for anchoring filaments in the lamina densa [97]. Other junctional sites resemble focal adhesions. Integrin $\alpha 3$b1 is concentrated in these sites of adhesion to the ECM. Other integrins, such as $\alpha 2\beta 1$ and $\alpha 5\beta 1$ and $\alpha 6\beta 1$, are found in the lateral domain, suggesting a role in cell-cell adhesion.

Fig. 4 is a schematic diagram of the organization of the various adhesive structures in epithelial cells.

8. CAM-Ig in cancer

Several N-CAM isoforms generated by splicing play different roles during development and in adults. The 120 kDa isoform is predominantly produced in normal adult issues whereas the 140 and 180 kDa isoforms are mostly expressed during development and have been shown to be produced in a number of cancers including pediatric tumors (Wilms' tumor, Ewing's sarcoma, neuroblastoma), melanoma and colon carcinoma. In non-small cell lung carcinoma, poor prognosis is associated with the expression of a polysialylated form of N-CAM [98]. Polysialylated N-CAM is generally produced during development, particularly in the nervous system, and it reduces the adhesion of N-CAM due to its negatively charged polymer [99,100]. In one clinical case, a peripheral, aggressive T-cell lymphoma was found to have metastasized in the brain, perhaps as a consequence of N-CAM expression [101]. However, N-CAM levels are low in a number of pancreatic and colon carcinomas. In an experimental murine model of pancreatic cancer induced by the SV40 T-antigen produced under the control of an insulin promoter, pancreatic tumors established in N-CAM-deficient mice were found to be highly metastatic. Recent studies have

shown that N-CAM forms heterotrimeric complexes at the cell surface with FGFR-4 and N-cadherin. These complexes are involved in signaling pathways leading to an increase in the level of interaction with the extracellular matrix. It has been suggested that this mechanism is responsible for stabilization of the cancer cells at the primary site. The loss of just one N-CAM allele is sufficient to induce the metastasis of pancreatic carcinoma cells [102]. Nr-CAM, a closely related member of the L1/Ng-CAM family that is overproduced in a number of carcinomas and in glioma and melanoma, was recently reported to induce malignancy in NIH–3T3 cells. The mechanism underlying the transformation of these fibroblast cells into tumorigenic cells is unclear. However, the Nr-CAM gene is a direct target of the Wnt-β-catenin signaling pathway, which is involved in oncogenesis (see Section 4.1). Nr-CAM overproduction may increase the motility of cancer cells, reproducing a phenomenon previously reported for neurite outgrowth [103].

Many studies have emphasized the role of MUC-18 and I-CAM-1 in melanoma progression [104]. These two molecules are produced in melanoma during the phase of radial growth in the skin. Their production increases during the vertical growth phase, a stage often associated with the formation of distant metastases. The mechanism by which I-CAM-1 and MUC-18 promote invasion and metastasis may be linked to their ability to mediate interactions with endothelial cells. This favors intravasation into blood vessels, promoting aggregation with leukocytes, thereby protecting melanoma cells in the bloodstream. One of the best known markers of carcinoma is the CEA antigen [105]. This adhesion molecule, when detected in the blood, indicates the presence of a carcinoma. The mechanism by which the CEA antigen promotes tumor progression is unknown. The CEA antigen is an intercellular adhesion molecule that has been shown to promote motility by interacting with the stromal cells surrounding carcinoma cells; this situation is reminiscent of the behavior of Nr-CAM, MUC-18 and I-CAM-1.

It remains to be seen how adhesive these molecules prove to be in transformed cells. It will be of particular importance to study the partner with which they interact in *cis*. It will also be vital to determine semi-quantitatively the strength of the adhesion mediated by CAM-Ig, and to compare adhesive behavior involving CAM-Ig with that involving cadherins.

9. Cadherins in cancer

The deregulation of various adhesion mechanisms is clearly associated with the progression of many different types of cancer towards a more malignant phenotype. One of the best examples of this is melanoma. Melanoma cells are produced by transformation of melanocytes in the skin. Melanocytes reside in the epidermis and maintain close interactions with keratinocytes by means of homophilic E-cadherin interactions. Following transformation and the progressive growth of the tumor, E-cadherin is lost from the cell surface and MUC18, a member of the Ig superfamily, appears in its place in melanoma cells invading the underlying dermis [104]. The repertoire of cell surface integrins is also modified, with a significant increase in the amount of $\alpha v\beta 3$ integrin. Changes in the expression patterns of these three adhesion molecules may will contribute to the early dissemination of cells in the dermis and their subsequent progression to form distant metastases, a situation frequently encountered in cases of melanoma and which is generally considered to indicate an extremely poor prognosis.

Carcinomas, which account for more than 90% of all tumors, are derived from epithelial cells. The adhesive status of epithelial cells is progressively modified throughout progression from an early adenoma to the invasive carcinoma stage. Epithelial cell polarity is lost and disorganized assemblies of cells, contrasting with their normal counterparts, gradually appear, partly due to changes in various adhesive processes.

E-cadherin, the prototype epithelial cell adhesion molecule was initially suspected to be downregulated in dedifferentiated carcinoma cells. An inverse correlation was found between the amount of E-cadherin expressed at the cell surface and the loss of epithelial cell polarity and between E-cadherin levels and the acquisition of an invasive phenotype, as assessed *in vitro* in three-dimensional collagen gels or in more complex extracellular matrices [106]. A similar correlation was found *in vivo* but these tumors often appear heterogeneous, with an area of carcinoma cells displaying a dedifferentiated phenotype associated with carcinoma cell islands forming glandular structures. A decrease in E-cadherin levels has often been found to be associated with a poor prognosis.

The mechanisms by which E-cadherin is downregulated in carcinoma and melanoma remained exclusive until recently. Several different mechanisms are responsible for the loss of E-cadherin [107]. The E-cadherin gene may be lost by gene deletion. A loss of heterozygosity is often observed at the E-cadherin locus (16q21). However, the loss of one allele is rarely associated with inactivating mutations in the other allele. This situation is found only in 50% of diffuse gastric carcinomas and in a fraction of lobular carcinomas of the breast [108,109]. In both cases, the carcinoma cells often acquire a fibroblast-like morphology. In most breast carcinomas of the ductal invasive type, transcription of the E-cadherin gene is downregulated by epigenetic mechanisms, either by hypermethylation of the promoter [110] or by specific repressors identified only very recently. The prototype of this group of repressors is Snail, a zinc finger nuclear protein that binds E2 boxes in the proximal region of the E-cadherin promoter [111]. An inverse correlation was found between levels of E-cadherin and of Snail transcripts in a number of carcinoma cell lines. A similar trend was observed in preliminary analyses of breast carcinomas *in vivo*, [112]. Other E-cadherin repressors have been identified that may also contribute to the extinction of the E-cadherin gene transcription

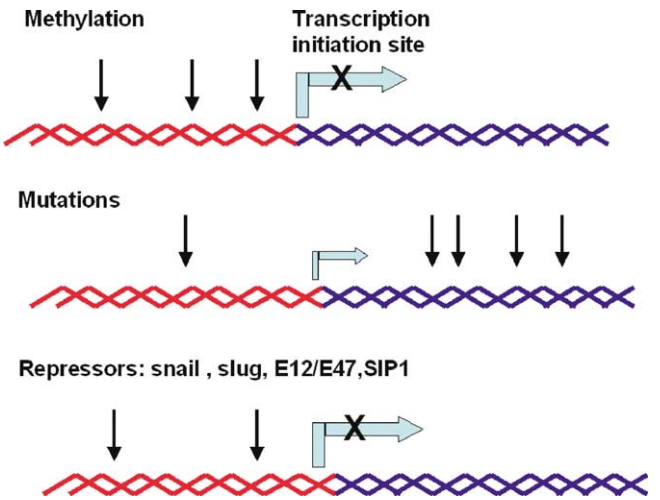

Fig. 5. A summary of the multiple mechanisms involved in E-cadherin down-regulation.

[113,114]. These very recent findings are consistent with many previous studies showing that E-cadherin plays an essential role as a caretaker of the epithelial state. The loss of E-cadherin may be temporary. Epigenetic control over the expression of the E-cadherin gene by means of hypermethylation or transcriptional repressors would make it possible for E-cadherin to be produced later, possibly in the primary and in metastatic tumors, accounting for some of the observed heterogeneity in E-cadherin production. A new mechanism involving the endocytosis and subsequent degradation of E-cadherin has been shown to be mediated in part by Hakai, an E3 ubiquitin ligase [115]. Interestingly, a number of secondary tumors display de novo E-cadherin expression. The transient loss of E-cadherin probably favors invasion by solitary cells and the subsequent dissemination of these cells via blood or lymph vessels. Micrometastases in the lymph nodes or bone marrow often consist of solitary cells devoid of E-cadherin, whereas the formation of a compact mass of carcinoma cells at the metastatic site would be favored by the resumption of E-cadherin production.

Fig. 5 summarizes the multiple mechanisms involved in E-cadherin down-regulation. Promoter transcribed region of the gene (red and blue respectively). Methylation and transcriptional repressors can block transcription while some mutations allow transcription although these transcripts may not be translated into functional proteins.

The distribution of desmosomes in the various phases of progression has not been determined precisely. It is important to establish whether desmosomes form in the absence of E-cadherin. In addition, it is now known that newly formed desmosomes are calcium-dependent, which may render more stable adhesive structures calcium-independent [116]. The targeted expression of a dominant negative construct of desmoplakin, the major component of the cytoplasm-dense plaque of desmosomes, was recently shown to inhibit the formation in keratinocytes of both desmosomes and adherens junctions. This demonstrates, for the first time, that desmosomes can also affect the E-cadherin-mediated formation of adherens junctions.

10. Role of cell-substratum adhesion in tumor progression

The role of cell-substratum adhesion has also been investigated in many tumor types. The composition of the extracellular matrix in tumors is very different from that in normal tissues. Tenascin, a large extracellular matrix protein with adhesive and anti-adhesive functional domains, is one of the first proteins to appear in the tumor stroma. However, as this protein is detected as early as the adenoma stage, it cannot be used as a prognostic marker [117].

Fibronectin was originally suspected to be absent from or present in different amounts in the tumor stroma. Specific isoforms are found in the stroma of tumors but seem to have no clear implications for tumor progression. A novel organization of fibronectin, known as supramolecular fibronectin, has been found to be associated with cancer cells [118]. Laminin 5 is currently the member of the laminin family thought most likely to promote tumor invasion. Experimental models have demonstrated this molecule to be important as a scatter factor and as a motogen [119–122]. It is found at the invasive front in colon and breast carcinomas.

Integrins, which are key receptors of extracellular matrix components, have been analyzed in some detail in various carcinomas [123,124]. Depending on the cell model of type of tumor used, certain integrins have been shown to be up- or down-regulated. Extensive studies of breast carcinoma have suggested a role for $\alpha 2\beta 1$ and $\alpha 6\beta 1$ [125]. The $\alpha 1\beta 1$ integrin is

specifically expressed by the basal cell layer in normal breast epithelia but is absent in breast carcinoma. The myoepithelial cells composing this layer gradually disappear during progression from the in situ stage to the invasive stage. In the in situ forms, lumen-derived carcinoma cells are still surrounded by myoepithelial cells [126]. The amount of $\alpha 2\beta 1$ integrin decreases during progression; there is some evidence that this integrin is involved in maintenance of the differentiated state. An increase in $\alpha 6$ integrin subunit levels seems to be correlated with a poor prognosis [127]. However, this study did not distinguish between $\alpha 6\beta 1$ and $\alpha 6\beta 4$. More recent studies have shown that $\alpha 6$b1 interacts with tyrosine kinase surface receptors, such as EGF or IGF1 receptors, to promote growth and survival during dissemination and at metastatic sites. PI3K and Rac may act as downstream effectors. The epithelial cell-specific integrin $\alpha 6\beta 4$ has been shown to be upregulated in some invasive carcinomas, including breast cancers [128]. $\alpha 6\beta 4$ may be released from hemidesmosomes by the action of a growth/scatter factor that induces an epithelial-mesenchymal transition. $\alpha 6\beta 4$ may then be recruited at other cell-substratum contacts in lamellipodia, in which this integrin associates with actin microfilaments. Its interaction with laminin 1 may increase the motility of carcinoma cells. The traction force exerted by $\alpha 6\beta 4$ is independent of the $\beta 1$-type integrins engaged in adhesion. The mechanism by which this integrin stimulates motility and invasion is unknown, but its association with several surface tyrosine kinase receptors indicates a cooperative effect between two signal transduction pathways. The protooncogene c-met cooperates with $\alpha 6\beta 4$ in the invasion and metastasis of carcinoma cells [129].

The pattern of expression of integrins has also been analyzed extensively in prostate cancers [130]. $\beta 1$ integrins are upregulated and redistributed during progression. However, the $\beta 1$c isoform, one of the splice variants, is downregulated. $\beta 3$ integrin is present in tumors whereas it is not expressed in normal prostate. Levels of $\alpha 6$, probably associated with $\beta 1$, are similar or may even be higher in lymph node metastases than in the primary tumor. These changes in the level of integrin expression must have a strong impact on the proliferation, migration and survival of cancer cells. Integrins, together with growth factor signaling, may activate various transduction pathways involving MAPK, AKT, Rac and other effectors.

Hyaluronan receptors, including CD44, have been implicated in the migration of tumor cells. CD44 is encoded by a gene with 18 exons, 10 of which are subject to alternative splicing. Numerous protein isoforms have been detected in normal and transformed epithelial cells. In one carcinoma model, CD44v6 was considered to promote metastasis. Another variant, CD4v10, may bind to hyaluronic acid with lower affinity than the standard form of CD44. Such a variant would facilitate cell migration in collagen-rich matrices. The expression of CD44v3 is correlated with a poor prognosis in breast cancers. However, it is thought that this variant is involved in the binding of growth factors controlling the bioavailability of mitogens to tumor cells [131].

11. Concluding remarks

Adhesion has long been thought to be affected during the malignant transformation of cells. In 1944, Coman showed that the mechanical dissociation of carcinoma cell doublets was easier than that of normal cell doublets. Many adhesive systems have since been identified. Cadherin-mediated adhesion by means of defined structures organized as adherens junctions and desmosomes is probably the principal mechanism ensuring epithelial cell stability. The cadherin-mediated adhesion system is responsible for the establishment of cell polarity, which culminates in the formation of distinct apical and basolateral domains separated by tight junctions. Perhaps equally important in epithelial cells is the assembly of basement membranes interacting with the $\alpha 6\beta 4$ integrin, the adhesive receptor of the hemidesmosomes. Studies on mechanisms governing epithelial cell plasticity during embryonic development are of the upmost importance to our understanding of the molecular and cellular processes involved in tumor progression. Recent studies on the mechanisms regulating E-cadherin expression in carcinomas have benefited considerably from previous work in *Drosophila* and on the embryos of several vertebrates.

The process of epithelial-mesenchymal transition (EMT) in gastrulation and subsequently in neural crest ontogeny involves mechanisms that are also activated *in vitro* in normal and carcinoma epithelial lines undergoing EMT. In the diagram summarizing the signal transduction involved in EMT, the parallel between gastrulation and bladder carcinoma EMT is striking [132]. Ongoing studies in many laboratories have clearly shown that there is cooperative signaling between growth factor receptors, integrins and cadherins in adhesion complexes.

Fig. 6 shows that similar EMT mechanisms operate in development and in the progression of carcinoma. Activation of tyrosine kinase surface receptors and integrins can induce a morphological transition from an epithelial to a mesenchymal state. The ras pathway can cooperate with other pathways to induce EMT and motility.

It is likely that, in tumor progression in vivo, most of the growth factors and ECM components involved are provided by the stromal cells that intermingle with malignant cells. Although the genetic basis of cancer has been firmly established, it is widely accepted that the microenvironment of the tumor affects tumor progression. One well-known case concerns TGFβ, which acts as a growth factor inhibitor in normal skin epithelial cells but enters into positive cooperation with the ras-activated pathway in cancers, increasing malignancy, in part through an EMT program. The remarkable transition of squamous carcinoma to spindle cell carcinoma provides a clear-cut example of the way in which adhesion mechanisms are among the primary effectors

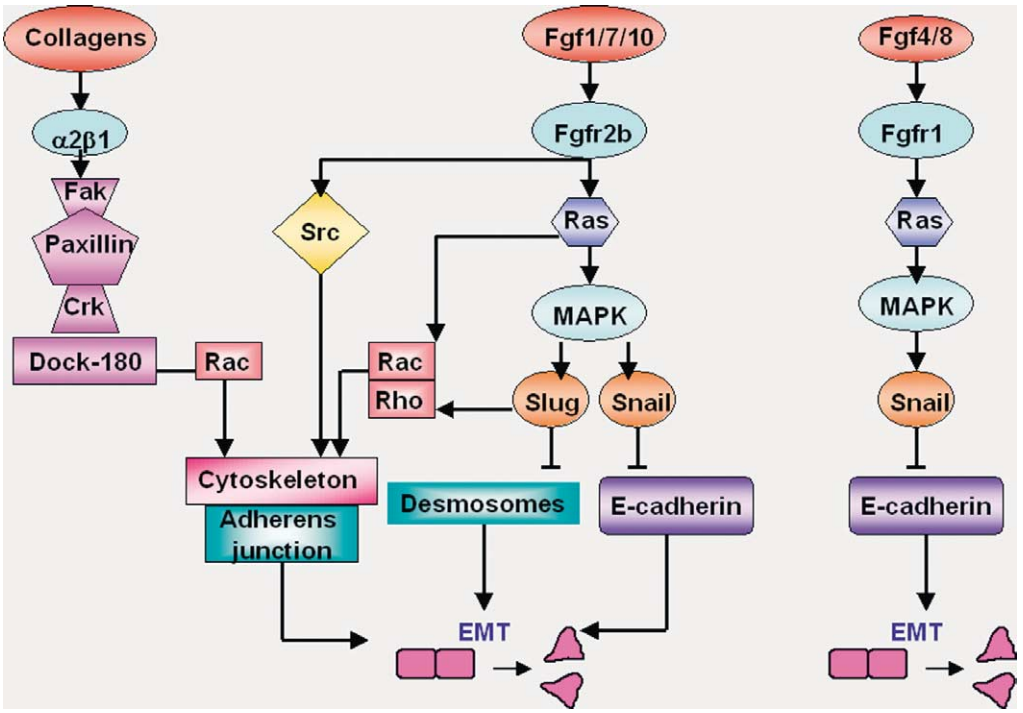

Fig. 6. Similar EMT mechanisms operate in development and in the progression of carcinoma.

of cancer [133]. Current knowledge concerning the signal transduction mechanisms regulating adhesion in cancer are already sufficiently advanced for new cancer treatment strategies to be proposed.

References

[1] G.M. Edelman, W.J. Gallin, A. Delouvee, B.A. Cunningham, J.P. Thiery, Proc. Nat. Acad. Sci. USA 80 (1983) 4384.
[2] P.S. Galtsoff, J. Exp. Morphol. 42 (1925) 223.
[3] P.L. Townes, J. Holtfreter, J. Exp. Zool. 128 (1955) 53.
[4] M.S. Steinberg, J. Exp. Zool. 173 (1970) 395.
[5] R.A. Foty, C.M. Pfleger, G. Forgacs, M.S. Steinberg, Development 122 (1996) 1611.
[6] R.E. Hausman, A.A. Moscona, Exp. Cell Res. 119 (1979) 191.
[7] R.W. Sperry, Proc. Nat. Acad. Sci. USA 50 (1963) 703.
[8] A.S.G. Curtis, Am. Naturalist 94 (1960) 37.
[9] J.P. Thiery, R. Brackenbury, U. Rutishauser, G.M. Edelman, J. Biol. Chem. 252 (1977) 6841.
[10] F. Hyafil, C. Babinet, F. Jacob, Cell 26 (1981) 447.
[11] B.A. Imhof, H.P. Vollmers, S.L. Goodman, W. Birchmeier, Cell 35 (1983) 667.
[12] M. Takeichi, J. Cell Biol. 75 (1977) 464.
[13] R. Schuh, et al., Proc. Nat. Acad. Sci. USA 83 (1986) 1364.
[14] W.J. Gallin, G.M. Edelman, B.A. Cunningham, Proc. Nat. Acad. Sci. USA 80 (1983) 1038.
[15] W.J. Gallin, B.C. Sorkin, G.M. Edelman, B.A. Cunningham, Proc. Nat. Acad. Sci. USA 84 (1987) 2808.
[16] M. Takeichi, Development 102 (1988) 639.
[17] A. Nose, A. Nagafuchi, M. Takeichi, EMBO J. 6 (1987) 3655.
[18] J.J. Hemperly, B.A. Murray, G.M. Edelman, B.A. Cunningham, Proc. Nat. Acad. Sci. USA 83 (1986) 3037.
[19] T. Kreis, T. Vale, Guidebook to the Extracellular Matrix Anchor, and Adhesion Proteins, 2nd edition, Oxford University Press, New York, 1999.
[20] M.J. Bastiani, A.L. Harrelson, P.M. Snow, C.S. Goodman, Cell 48 (1987) 745.
[21] G.M. Edelman, Immunol. Rev. 100 (1987) 11.
[22] L. Shapiro, et al., Nature 374 (1995) 327.
[23] T.J. Boggon, et al., Science 296 (2002) 1308.
[24] T.A. Springer, Cell 76 (1994) 301.
[25] R.A. Worthylake, K. Burridge, Curr. Opin. Cell Biol. 13 (2001) 569.

[26] M. Balzar, et al., Mol. Cell Biol. 21 (2001) 2570.
[27] B. Obrink, Curr. Opin. Cell Biol. 9 (1997) 616.
[28] M. Tessier-Lavigne, C.S. Goodman, Science 274 (1996) 1123.
[29] B.P. Liu, S.M. Strittmatter, Curr. Opin. Cell Biol. 13 (2001) 619.
[30] S. Artavanis-Tsakonas, M.D. Rand, R.J. Lake, Science 284 (1999) 770.
[31] S. Weijzen, et al., Nat. Med. 8 (2002) 979.
[32] M. Bailly, J. Condeelis, Nat. Cell Biol. 4 (2002) E292.
[33] E.D. Hay, Cell Biology of Extracellular Matrix, Plenum Press, New York, 1991.
[34] M. Baron, et al., Biochemistry 31 (1992) 2068.
[35] B.O. Smith, et al., Biochemistry 33 (1994) 2422.
[36] D.J. Leahy, I. Aukhil, H.P. Erickson, Cell 84 (1996) 155.
[37] R. Timpl, et al., J. Biol. Chem. 254 (1979) 9933.
[38] C.K. Miranti, J.S. Brugge, Nat. Cell Biol. 4 (2002) E83.
[39] M. Bernfield, et al., Annu. Rev. Cell Biol. 8 (1992) 365.
[40] A. Woods, J.R. Couchman, Curr. Opin. Cell Biol. 13 (2001) 578.
[41] A.J. Day, G.D. Prestwich, J. Biol. Chem. 277 (2002) 4585.
[42] M. Ekblom, M. Falk, K. Salmivirta, M. Durbeej, P. Ekblom, Ann. New York Acad. Sci. 857 (1998) 194.
[43] M. Durbeej, K.P. Campbell, Curr. Opin. Genet. Dev. 12 (2002) 349.
[44] A.S.G. Curtis, J.M. Lackie, Measuring Cell Adhesion, Wiley, Chichester, 1991.
[45] A. Pierres, A.M. Benoliel, P. Bongrand, Cell Adhes. Commun. 5 (1998) 375.
[46] A.F. Williams, A.N. Barclay, Annu. Rev. Immunol. 6 (1988) 381.
[47] E.S. Lander, et al., Nature 409 (2001) 860.
[48] J.C. Venter, et al., Science 291 (2001) 1304.
[49] J.P. Thiery, in: T. Kreis, R. Vale (Eds.), Guide Book to the Extracellular Matrix, Anchor and Adhesion Proteins, Oxford University Press, New York, 1999.
[50] D. Schmucker, et al., Cell 101 (2000) 671.
[51] T. Brumendorf, in: T. Kreis, R. Vale (Eds.), Guidebook to the Extracellular Matrix, Anchor and Adhesion Proteins, Oxford University Press, New York, 1999, p. 568.
[52] T. Mizuno, et al., Mol. Cell Neurosci. 18 (2001) 119.
[53] T. Brummendorf, V. Lemmon, Curr. Opin. Cell Biol. 13 (2001) 611.
[54] M. Takeichi, Ann. Rev. Biochem. 59 (1990) 237.
[55] S. Suzuki, K. Sano, H. Tanihara, Cell Regul. 2 (1991) 261.
[56] F. Nollet, P. Kools, F. Van Roy, J. Mol. Biol. 299 (2000) 551.
[57] B.D. Angst, C. Marcozzi, A.I. Magee, J. Cell Sci. 114 (2001) 629.
[58] B. Nagar, M. Overduin, M. Ikura, J.M. Rini, Nature 380 (1996) 360.
[59] K. Tamura, W.S. Shan, W.A. Hendrickson, D.R. Colman, L. Shapiro, Neuron 20 (1998) 1153.
[60] O. Pertz, et al., EMBO J. 18 (1999) 1738.
[61] S. Sivasankar, B. Gumbiner, D. Leckband, Biophys. J. 80 (2001) 1758.
[62] E. Perret, et al., EMBO J. 21 (2002) 2537.
[63] M. Ozawa, H. Baribault, R. Kemler, EMBO J. 8 (1989) 1711.
[64] A.H. Huber, W.J. Nelson, W.I. Weis, Cell 90 (1997) 871.
[65] P.Z. Anastasiadis, A.B. Reynolds, Curr. Opin. Cell Biol. 13 (2001) 604.
[66] V. Korinek, et al., Science 275 (1997) 1784.
[67] M. Shtutman, et al., Proc. Nat. Acad. Sci. USA 96 (1999) 5522.
[68] T. Brabletz, A. Jung, S. Dag, F. Hlubek, T. Kirchner, Am. J. Pathol. 155 (1999) 1033.
[69] M. Peifer, P. Polakis, Science 287 (2000) 1606.
[70] J.B. Xanthos, et al., Development 129 (2002) 4027.
[71] P. Polakis, Curr. Biol. 12 (2002) R499.
[72] R. Fodde, R. Smits, H. Clevers, Nat. Rev. Cancer 1 (2001) 55.
[73] D.R. Garrod, A.J. Merritt, Z. Nie, Curr. Opin. Cell Biol. 14 (2002) 537.
[74] C. Jamora, E. Fuchs, Nat. Cell Biol. 4 (2002) E101.
[75] K. Sano, et al., EMBO J. 12 (1993) 2249.
[76] M. Frank, R. Kemler, Curr. Opin. Cell Biol. 14 (2002) 557.
[77] T. Usui, et al., Cell 98 (1999) 585.
[78] M.A. Buratovich, P.J. Bryant, Genetics 147 (1997) 657.
[79] R. Hynes, Cell 110 (2002) 673.
[80] J.P. Xiong, et al., Science 296 (2002) 151.
[81] B. Yan, D.A. Calderwood, B. Yaspan, M.H. Ginsberg, J. Biol. Chem. 276 (2001) 28164.
[82] V. Martel, et al., J. Biol. Chem. 276 (2001) 21217.
[83] E. Zamir, B. Geiger, J. Cell Sci. 114 (2001) 3583.
[84] B. Geiger, A. Bershadsky, Curr. Opin. Cell Biol. 13 (2001) 584.
[85] E. Cukierman, R. Pankov, D.R. Stevens, K.M. Yamada, Science 294 (2001) 1708.

[86] F.G. Giancotti, E. Ruoslahti, Science 285 (1999) 1028.
[87] B. Geiger, A. Bershadsky, R. Pankov, K.M. Yamada, Nat. Rev. Mol. Cell Biol. 2 (2001) 793.
[88] H. Park, Y. Kim, Y. Lim, I. Han, E.S. Oh, J. Biol. Chem. 277 (2002) 29730.
[89] U. Gunthert, et al., Cell 65 (1991) 13.
[90] A. Gautreau, P. Poullet, D. Louvard, M. Arpin, Proc. Nat. Acad. Sci. USA 96 (1999) 7300.
[91] H. Mori, et al., EMBO J. 21 (2002) 3949.
[92] M. Seiki, Curr. Opin. Cell Biol. 14 (2002) 624.
[93] S. Tsukita, M. Furuse, Curr. Opin. Cell Biol. 14 (2002) 531.
[94] I. Martin-Padura, et al., J. Cell Biol. 142 (1998) 117.
[95] Y. Takai, H. Nakanishi, J. Cell Sci. 116 (2003) 17.
[96] N.M. Kumar, N.B. Gilula, Cell 84 (1996) 381.
[97] M.F. Jonkman, J. Dermatol. Sci. 20 (1999) 103.
[98] F. Tanaka, et al., Cancer Res. 61 (2001) 1666.
[99] G.M. Edelman, B.A. Cunningham, J.P. Thiery, Wiley, New York, 1990.
[100] U. Rutishauser, Curr. Opin. Cell Biol. 8 (1996) 679.
[101] C.S. Chim, C.C. Lam, J.M. Nicholls, G.C. Ooi, Y.L. Kwong, J. Clin. Oncol. 20 (2002) 3742.
[102] U. Cavallaro, J. Niedermeyer, M. Fuxa, G. Christofori, Nat. Cell Biol. 3 (2001) 650.
[103] M.E. Conacci-Sorrell, et al., Genes. Dev. 16 (2002) 2058.
[104] J.P. Johnson, Cancer Metastasis Rev. 18 (1999) 345.
[105] N.L. Berinstein, J. Clin. Oncol. 20 (2002) 2197.
[106] J. Behrens, W. Birchmeier, Cancer Treat. Res. 71 (1994) 251.
[107] S. Hirohashi, Am. J. Pathol. 153 (1998) 333.
[108] G. Berx, K.F. Becker, H. Hofler, F. Van Roy, Hum. Mutat. 12 (1998) 226.
[109] K.F. Becker, et al., Cancer Res. 54 (1994) 3845.
[110] C.W. Cheng, et al., Oncogene 20 (2001) 3814.
[111] A. Cano, et al., Nat. Cell Biol. 2 (2000) 76.
[112] M.J. Blanco, et al., Oncogene 21 (2002) 3241.
[113] M.A. Perez-Moreno, et al., J. Biol. Chem. 17 (2001) 17.
[114] J. Comijn, et al., Mol. Cell 7 (2001) 1267.
[115] Y. Fujita, et al., Nat. Cell Biol. 4 (2002) 222.
[116] S. Wallis, et al., Mol. Biol. Cell 11 (2000) 1077.
[117] E.J. Mackie, Int. J. Biochem. Cell Biol. 29 (1997) 1133.
[118] A. Morla, Z. Zhang, E. Ruoslahti, Nature 367 (1994) 193.
[119] M. Grassi, G. Moens, P. Rousselle, J.P. Thiery, J. Jouanneau, J. Cell Sci. 112 (1999) 2511.
[120] M.A. Deugnier, et al., J. Cell Biol. 159 (2002) 453.
[121] N. Koshikawa, G. Giannelli, V. Cirulli, K. Miyazaki, V. Quaranta, J. Cell Biol. 148 (2000) 615.
[122] I. Sordat, et al., Int. J. Cancer 88 (2000) 708.
[123] J.A. Varner, D.A. Cheresh, Curr. Opin. Cell Biol. 8 (1996) 724.
[124] C. Brakebusch, D. Bouvard, F. Stanchi, T. Sakai, R. Fassler, J. Clin. Invest. 109 (2002) 999.
[125] L.M. Shaw, J. Mammary Gland Biol. Neoplasia 4 (1999) 367.
[126] M.A. Deugnier, J. Teuliere, M.M. Faraldo, J.P. Thiery, M. Glukhova, Breast Cancer Res. 4 (2002) 224.
[127] K. Friedrichs, et al., Cancer Res. 55 (1995) 901.
[128] A.M. Mercurio, I. Rabinovitz, L.M. Shaw, Curr. Opin. Cell Biol. 13 (2001) 541.
[129] L. Trusolino, A. Bertotti, P.M. Comoglio, Cell 107 (2001) 643.
[130] M. Fornaro, T. Manes, L.R. Languino, Cancer Metastasis Rev. 20 (2001) 321.
[131] L.Y. Bourguignon, J. Mammary Gland Biol. Neoplasia 6 (2001) 287.
[132] J.P. Thiery, Nat. Rev. Cancer 2 (2002) 442.
[133] M. Oft, R.J. Akhurst, A. Balmain, Nat. Cell Biol. 4 (2002) 487.