CELL ADHESION AND SIGNALLING BY CADHERINS AND IG-CAMS IN CANCER

Ugo Cavallaro‡ and Gerhard Christofori**

In addition to their adhesive functions, cell-adhesion molecules modulate signal-transduction pathways by interacting with molecules such as receptor tyrosine kinases, components of the WNT signalling pathway and RHO-family GTPases. So, changes in the expression of cell-adhesion molecules affect not only the adhesive repertoire of a cell, but also its signal-transduction status. Conversely, signalling pathways can modulate the function of cell-adhesion molecules, altering the interactions between cells and their environment. Recent experimental evidence indicates that such processes have a crucial role in tumour progression, in particular during invasion and metastasis.

TIGHT JUNCTIONS (TJs). Specialized intercellular junctions that are formed by several proteins in which two plasma membranes form a sealing gasket around a cell (also known as zonula occludens). Prevent fluid moving through the intercellular gap and lateral diffusion of membrane proteins between the apical and basolateral membranes.

*Institute of Biochemistry and Genetics, Department of Clinical-Biological Sciences, University of Basel, Switzerland.
‡IFOM-FIRC Institute of Molecular Oncology, Milan, Italy.
Correspondence to G.C.
e-mail: gerhard.christofori@unibas.ch
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The metastatic dissemination of tumour cells is the primary cause of death in patients with cancer. So, understanding the molecular mechanisms that underlie tumour progression, local invasion and the formation of tumour metastases represents one of the great challenges in exploratory cancer research. As early as 1914, Theodor Boveri recognized the importance of changes in the adhesion of tumour cells to the development of cancer[1]. The observation that malignant tumour cells leave the primary tumour to disseminate to distant organs and that tumour cells show marked changes in their interaction with extracellular-matrix components has led to the notion that changes in cell–cell and cell–matrix adhesion coincide with tumour progression.

Recent experimental results indicate that, as well as mediating intercellular and cell–matrix interactions, cell-adhesion molecules also directly modulate signal transduction. Changes in the expression or function of cell-adhesion molecules can therefore contribute to tumour progression both by altering the adhesion status of the cell and by affecting cell signalling. Cell-adhesion molecules of various classes and functions, including cadherins, immunoglobulin-like cell-adhesion molecules (Ig-CAMs), the hyaluronan receptor CD44 and integrins, can interact with and modulate several signalling pathways. Conversely, signalling molecules can directly affect the function of adhesion molecules, leading to changes in cell–cell and cell–matrix interactions. Loss of epithelial (E)-cadherin, gain of mesenchymal cadherins and changes in the expression of Ig-CAMs during the progression of many cancer types exemplify the functional implications of this. So, what are the molecular mechanisms of cadherin- and Ig-CAM-mediated signal transduction; how do signalling pathways affect the adhesive properties of a cell; and what is the relevance of these processes to tumour progression?

Adhesion by cadherins and Ig-CAMs

The cadherins. Adhesion between vertebrate cells is generally mediated by three types of adhesion junction: TIGHT JUNCTIONS (TJs), ADHERENS JUNCTIONS (AJs), and DESMOSOMES. Together they constitute the intercellular junctional complex, which has an important role in defining the physiological function of a cell; that is, they define whether and how a cell will be integrated in functional structures, such as organ epithelia or stroma. Cadherins are the principal components of AJs and desmosomes, and cluster at sites of cell–cell contact in most solid tissues[2,3] (FIG. 1). The cadherin superfamily consists of classical cadherins, which are the main mediators of calcium-dependent cell–cell adhesion, and non-classical cadherins, which include

Summary

- Cell-adhesion molecules of the cadherin and immunoglobulin-like cell-adhesion molecule (Ig-CAM) superfamilies not only exert their functions by mediating cell–cell and cell–matrix adhesion, but also by directly eliciting signals that are involved in tissue morphogenesis and tumour progression.
- In addition, signalling molecules are also able to modulate the adhesion status of the cell by acting on cell-adhesion molecules themselves, or on other components of signalling complexes.
- The function of epithelial (E)-cadherin is altered in most epithelial tumours during the progression to tumour malignancy. E-cadherin function can be disrupted by various genetic and epigenetic mechanisms, including modulation by signalling molecules.
- Loss of E-cadherin function elicits active signals that support tumour-cell migration, invasion and metastatic dissemination.
- In several cancer types, loss of E-cadherin function is accompanied by the gain of expression of mesenchymal cadherins, for example, neuronal (N)-cadherin and cadherin-11, in a process that is known as the cadherin switch.
- N-cadherin interacts with members of the fibroblast growth factor receptor (FGFR) family, thereby inducing pro-migratory and invasive signalling cascades.
- Neural CAM (NCAM) also associates with FGFRs. Loss of NCAM function during tumour progression affects cell–matrix adhesion through the loss of FGFR-induced, integrin-mediated cell–matrix adhesion.
- Several other members of the cadherin and Ig-CAM families interact with signalling molecules, thereby modulating physiological and pathological processes.

desmosomal cadherins and the recently discovered large subfamily of protocadherins, which are implicated in neuronal plasticity. The functional role of non-classical cadherins in tumour progression is unknown, so in this review we will focus on classical cadherins.

Classical cadherins are a family of single-span transmembrane-domain glycoproteins that function as specific cell–cell adhesion molecules. Cadherin-mediated cell–cell adhesion is accomplished by homophilic protein–protein interactions between two cadherin molecules at the surface of the respective cells (FIG. 1). This interaction is thought to be mediated by interactions between the histidine–alanine–valine (HAV) domains and between tryptophan residues and hydrophobic pockets in the most amino-terminal cadherin domains (FIG.1). How the remaining cadherin domains contribute to the stabilization of cell adhesion, and whether the cadherin molecules interact with each other in a zipper-like fashion, are matters of debate. Recent structural analysis by electron tomography indicates that individual cadherin molecules form groups and interact through their tips in a highly flexible manner[4]. Cadherins show an exquisite specificity in their homophilic interactions by almost exclusively binding the same type of cadherin on another cell.

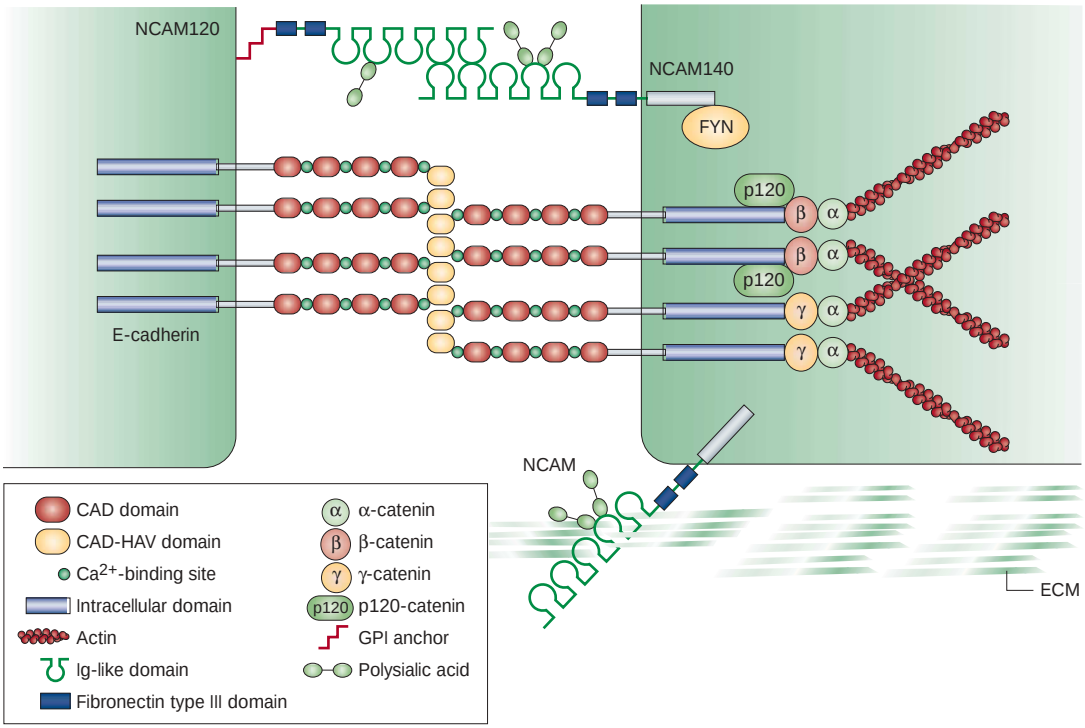


Figure 1 | **Model of cell–cell and cell–matrix adhesion by cadherins and NCAM.** Epithelial (E)-cadherin molecules that are expressed on the plasma membranes of adjacent cells probably interact in a zipper-like fashion, although this is a matter of debate (see main text). The most amino-terminal cadherin (CAD) domain on each E-cadherin molecule contains the histidine–alanine–valine (HAV) motif that is thought to interact with E-cadherin molecules of adjacent cells. Recent structural analysis indicates that cadherin molecules interact through their tips in *cis* and in *trans* in a highly flexible manner. The cytoplasmic cell-adhesion complex (CCC), consisting of α-catenin, β-catenin, γ-catenin (plakoglobin) and p120-catenin, links E-cadherin homodimers to the actin cytoskeleton. Neural cell-adhesion molecule (NCAM), a member of the immunoglobulin (Ig)-like CAM superfamily, interacts through its first two Ig-like domains in a homophilic manner with NCAM molecules on neighbouring cells. Different NCAM isoforms exist, which are either linked to the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor (NCAM120) or have transmembrane and cytoplasmic domains (NCAM140 and NCAM180). NCAM140 can associate through its cytoplasmic domain with the SRC family kinase FYN. NCAM also interacts with components of the extracellular matrix (ECM). The various isoforms of NCAM are differentially polysialylated, a post-translational modification that modulates their adhesive functions.

ADHERENS JUNCTIONS
(AJs). Specialized cell–cell junctions that are formed by cadherins and additional associated proteins into which actin filaments are inserted (also known as zonula adherens).

DESMOSOMES
Specialized cell junctions that are formed by desmosomal cadherins and additional associated proteins into which intermediate filaments are inserted. Also known as macula adherens junctions or spot desmosomes.

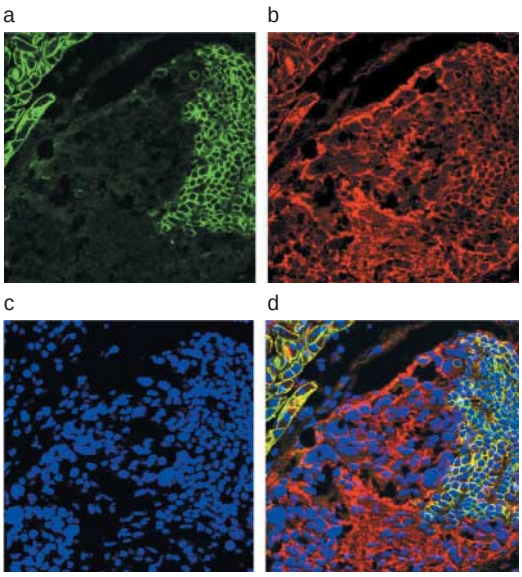


Figure 2 | **Loss of E-cadherin expression during tumour progression.** A tissue section of a β-cell tumour from a *Rip1Tag2* transgenic mouse (a model of pancreatic β-cell carcinogenesis) has been stained with antibodies against E-cadherin (**a**) and β-catenin (**b**), and with DAPI to visualize the nuclei (**c**). Tumour cells on the left-hand side of the tumour have lost E-cadherin expression, whereas the tumour cells on the right-hand side still express E-cadherin. Note the marked change of nuclear shape and downregulation of β-catenin concomitant with the loss of E-cadherin expression. **d** | Merged image of the stainings shown in **a**–**c**.

The intracellular domain of classical cadherins (which is lacking in non-classical cadherins and protocadherins) interacts with various catenin proteins to form the cytoplasmic cell-adhesion complex (CCC). β-catenin and γ-catenin (also known as plakoglobin) bind to the same conserved site at the carboxyl termini of classical cadherins in a mutually exclusive way, whereas another catenin, p120-catenin, interacts with several sites in the cytoplasmic tail, including the juxtamembrane region. β-catenin and γ-catenin bind directly to α-catenin, which links the CCC to the actin cytoskeleton. Without an intact CCC, cadherin-mediated strong cell–cell adhesion is compromised and, conversely, without cell–cell adhesion the CCC will not form. Recent detailed analysis of the potential protein–protein interaction partners of the cytoplasmic tail of cadherins and those of catenins has revealed a large number of additional interaction partners that also link the CCC to the microtubule network and to several signalling molecules[2,3].

The Ig-CAMs. Adhesion receptors of the immunoglobulin superfamily are expressed in a wide variety of cell types, including cells of the nervous system, leukocytes and epithelial and endothelial cells. This heterogeneous expression pattern implicates Ig-CAMs in many diverse biological processes, such as brain development, immune responses, tissue sorting, epithelial morphogenesis and the development of the vascular network, to name but a few[5]. Ig-CAMs are characterized by the presence of one or more Ig-like domains in their extracellular region (FIG. 1). In addition, the ectodomain of Ig-CAMs can contain various numbers of fibronectin type III (FNIII) repeats. Although most Ig-CAMs have a transmembrane domain and a cytoplasmic tail, some of them are linked to the cell surface by a glycosylphosphatidylinositol anchor (FIG. 1). The best-characterized biological function of Ig-CAMs is the support of cell–cell adhesion through their homophilic interactions in *trans*. However, Ig-CAMs can also exert heterophilic interactions, as different members of the Ig-CAM superfamily are known to bind to each other and even to other types of molecules, including components of the extracellular matrix. More recently, Ig-CAMs have also been reported to associate with various proteins on the membrane of the same cell, such as growth-factor receptors, integrins and cadherins. In addition, Ig-CAMs have several intracellular binding partners, ranging from effectors of signal-transduction pathways to cytoskeletal proteins. Taken together, these observations account for the broad spectrum of biological functions of Ig-CAMs, including cell adhesion, migration, signal transduction and the regulation of gene expression[6].

Loss of E-cadherin in tumorigenesis

Most human cancers originate from epithelial tissue. E-cadherin — the prototype member of the classical cadherin family — is the key player in inducing cell polarity and organizing an epithelium. In most, if not all, cancers of epithelial origin, E-cadherin-mediated cell–cell adhesion is lost concomitantly with progression towards tumour malignancy (FIG. 2). Although E-cadherin expression can still be found in differentiated tumours in patients, there is an inverse correlation between E-cadherin levels, tumour grade and patient mortality rates[7,8].

Based on the descriptive and functional data, it has been proposed that the loss of E-cadherin-mediated cell–cell adhesion is a prerequisite for tumour-cell invasion and metastasis formation[7]. Subsequently, several groups demonstrated that re-establishing the functional cadherin complex — for example, by forced expression of E-cadherin — resulted in a reversion from an invasive, mesenchymal phenotype to a benign, epithelial phenotype of cultured tumour cells[7,9]. Using the *RIP1TAG2* model of pancreatic β-cell carcinogenesis, our group has previously demonstrated that the loss of E-cadherin-mediated cell–cell adhesion (FIG. 2) is causally involved in the progression from adenoma to carcinoma *in vivo*[10]. Intercrossing *Rip1Tag2* mice with transgenic mice that maintain E-cadherin expression in β-cell tumour cells results in the arrest of tumour development at the adenoma stage, whereas expression of a dominant-negative form of E-cadherin induces early invasion and metastasis. These results show that the loss of E-cadherin-mediated cell adhesion is one rate-limiting step in the progression from adenoma to carcinoma and the subsequent formation of tumour metastases.

RIP1TAG2
A transgenic mouse line that expresses the simian virus 40 large T antigen (*Tag*) under the control of the rat insulin II promoter (*Rip*) in the β-cells of pancreatic islets of Langerhans. Carcinomas develop in the pancreatic islets by progression through characteristic tumour stages.

The loss of E-cadherin function during tumour progression can be caused by various genetic or epigenetic mechanisms. In patients with diffuse gastric cancer and lobular breast cancer, and at a lower incidence in thyroid, bladder and gynaecological cancers, the E-cadherin gene is mutated, leading to the expression of a non-functional protein[11]. Such mutations have also been found in families that are predisposed to the development of diffuse gastric cancer[12].

In most cases, E-cadherin expression is downregulated at the transcriptional level. The zinc-finger-containing proteins Snail, Slug and SIP1, and the helix–loop–helix transcription factor E12/E47 are important transcriptional repressors that bind to E2 boxes in the promoter of the E-cadherin gene and actively repress its expression[13–17]. Interestingly, MTA3, a recently identified component of the Mi-2/NuRD transcriptional co-represssor complex, links Snail expression to oestrogen-receptor (ER) activity: ER signalling upregulates MTA3 to repress Snail expression. In turn, the lack of Snail allows unimpeded E-cadherin expression, so preventing tumour invasion. This connection might be one of the several mechanisms linking positive ER status with good prognosis in patients with breast cancer[18]. As a direct consequence of transcriptional inactivation, the E-cadherin gene locus is epigenetically silenced by hypermethylation, leading to further downregulation of E-cadherin expression. For example, the E-cadherin promoter is hypermethylated in 83% of thyroid carcinomas and at comparably high levels in many other cancer types[19].

Proteolytic degradation of E-cadherin by matrix metalloproteases (MMPs) is another mechanism by which E-cadherin-mediated cell–cell adhesion can be ablated. A soluble 80-kDa form of E-cadherin, produced by the degradation of the full-length protein, is frequently found in cultured tumour cell lines and in tumour biopsy samples. This soluble form of E-cadherin promotes tumour-cell invasion by upregulating MMPs, such as MMP2, MMP9 and MMP14. Such ectodomain shedding of E-cadherin might have an active part in the invasive process during tumour progression[20]. A novel transmembrane protein, dysadherin, interferes with E-cadherin function by downregulating its protein levels, without affecting messenger RNA levels, and it induces the metastatic spread of tumour cells in XENOGRAFT TRANSPLANTATION experiments[21].

Tyrosine phosphorylation has been previously implicated in the regulation of cadherin function: receptor tyrosine kinases (RTKs; which are frequently activated in cancer cells), such as epidermal growth factor receptor (EGFR), hepatocyte growth factor receptor (c-MET) and fibroblast growth-factor receptor (FGFR), and the non-RTK SRC, phosphorylate E-cadherin, neuronal (N)-cadherin, β-catenin, γ-catenin and p120-catenin, resulting in the disassembly of the CCC and, with it, the disruption of cadherin-mediated cell–cell adhesion[22–24] (FIG. 3a). However, a functional implication of this mechanism in tumour progression remains to be shown.

One mechanism by which RTKs can disrupt the CCC is by targeting E-cadherin for degradation. On autophosphorylation, RTKs are often ubiquitylated by E3 LIGASES, such as c-CBL, which associate with phosphorylated RTKs, resulting in the degradation of the RTK by the PROTEASOME. Recently, Walter Birchmeier's group identified a novel c-CBL-related E3 ligase, known as Hakai (Japanese for destruction), that specifically binds and ubiquitylates tyrosine-phosphorylated E-cadherin (but not N-cadherin) and so earmarks it for endocytosis and proteasome-mediated degradation[23]. Conversely, β-catenin interacts with the low-molecular-weight protein tyrosine phosphatase (LMW-PTP), which counteracts tyrosine phosphorylation and promotes the stability of E-cadherin-mediated AJs[25]. How the balance between the two processes is regulated and what role they have in tumour progression remains to be investigated.

Insulin-like growth factor 2 (IGF2), a peptide growth factor that binds and activates the IGF1 receptor (IGF1R), is able to induce an EPITHELIAL–MESENCHYMAL transition (EMT), concomitant with the loss of E-cadherin function. Curiously, IGF1R has been found in a complex with E-cadherin and β-catenin and, following IGF2 stimulation, E-cadherin is internalized and degraded by an unknown mechanism. Subsequently, β-catenin translocates from the plasma membrane to the nucleus, resulting in the modulation of β-catenin–TCF target-gene expression (see below)[26]. In the *RipTag2* transgenic mouse model of pancreatic β-cell carcinogenesis, transgenic overexpression of *Igf1r* results in a marked acceleration of tumour progression from benign adenoma to invasive carcinoma, and to the formation of metastases concomitantly with the loss of E-cadherin function[27]. By contrast, IGF1R activation promotes the formation of E-cadherin-mediated AJs in MCF7 breast cancer cells[28]. The basis for these contradictory results remains unexplained.

Hepatocyte-growth-factor (HGF)-induced scattering and motility of epithelial cells is also mediated by an activated RTK: phosphorylation of components of the CCC by the activated c-MET RTK disrupts E-cadherin-mediated cell–cell adhesion, possibly by co-endocytosis of E-cadherin with c-MET[29]. E-cadherin has been reported to associate with c-MET at the basolateral membranes of polarized epithelial cells and in colon, breast and prostate cancer cell lines, further supporting the existence of functional crosstalk between RTKs and cell-adhesion molecules[30].

Mechanisms of E-cadherin signalling

Whereas the signals and molecules that are involved in the formation of E-cadherin-mediated cell–cell adhesion complexes have been extensively studied[3], the signals that are elicited by the loss of E-cadherin function during development and cancer progression are only just being elucidated. The observation that downregulation of E-cadherin function in most epithelial cell types results in a fundamental change in cellular phenotype (namely, a reduced cell polarity

XENOGRAFT TRANSPLANTATION
Transplantation of tissue or cells from one species to another. In cancer research, most xenografts are human cancer cell lines or human tumours that have been transplanted to immunodeficient rodents.

E3 LIGASE
The third enzyme in a series — the first two are designated E1 and E2 — that are responsible for ubiquitylation of target proteins. E3 enzymes provide platforms for binding E2 enzymes and specific substrates, thereby coordinating ubiquitylation of the selected substrates.

PROTEASOME
A 26S multiprotein complex that catalyses the breakdown of polyubiquitylated proteins.

EPITHELIAL–MESENCHYMAL TRANSITION
(EMT). Conversion from an epithelial to a mesenchymal phenotype, which is a normal process of embryonic development. In carcinomas, this transformation results in altered cell morphology, the expression of mesenchymal proteins and increased invasiveness.

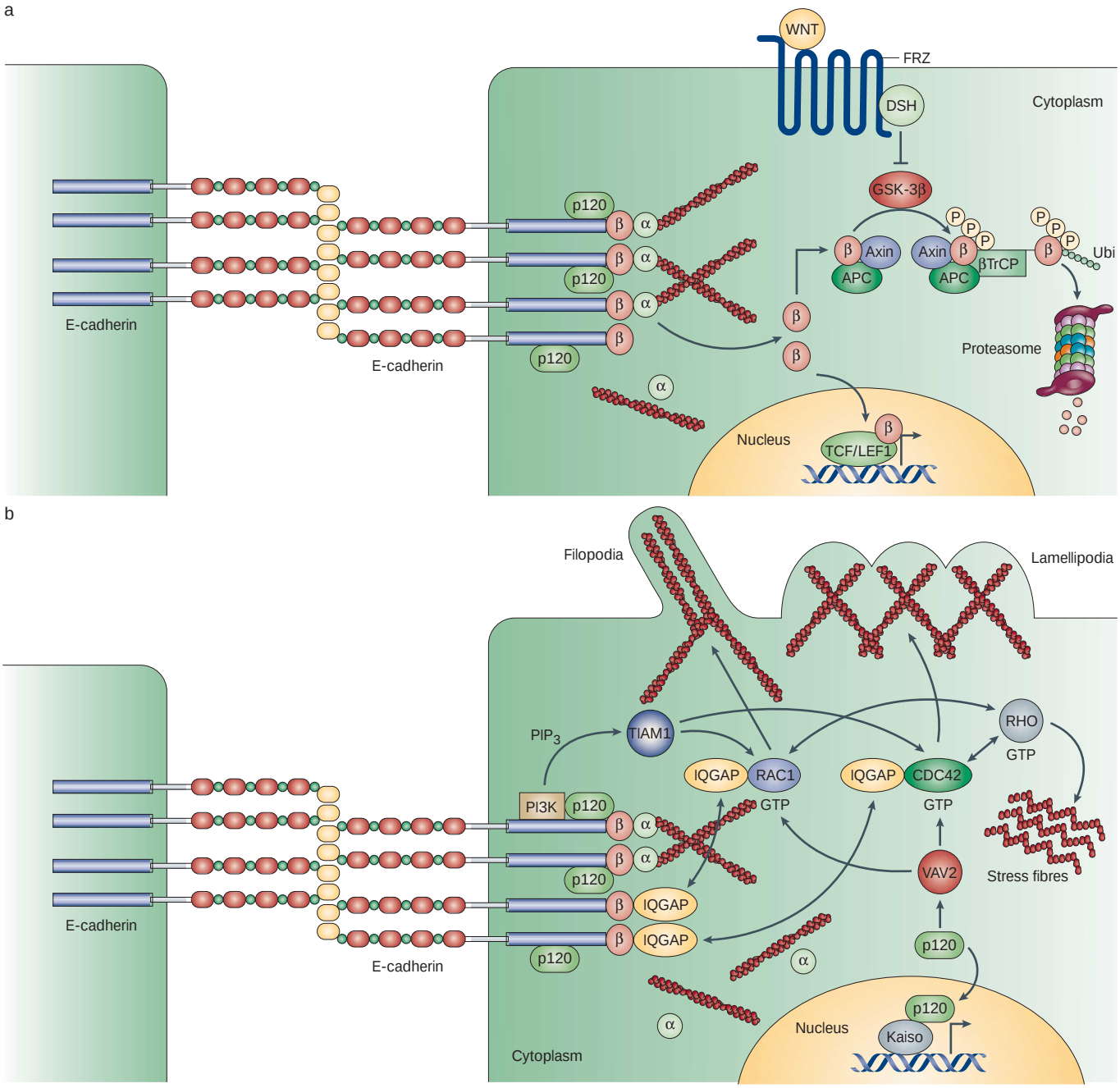


Figure 3 | **Potential signalling pathways affected by loss of E-cadherin function. a** | After loss of epithelial (E)-cadherin function and disassembly of the cytoplasmic cell-adhesion complex (CCC), catenins are released and accumulate in the cytoplasm. β-Catenin (β) is then sequestered by the adenomatous polyposis coli (APC)–axin–glycogen synthase kinase 3β (GSK-3β) complex and phosphorylated by GSK-3β. Phosphorylated β-catenin is specifically bound by βTrCP, a subunit of the E3 ubiquitin-ligase complex, which ubiquitylates β-catenin and thereby earmarks it for rapid proteosomal degradation. However, on activation of the WNT signalling pathway, GSK-3β is repressed and β-catenin is no longer phosphorylated. It translocates to the nucleus where, together with the TCF/LEF1 transcription factors, it modulates the expression of several target genes that are known to be involved in cell proliferation and tumour progression. **b** | Cytoplasmic p120-catenin (p120) activates the RHO-family GTPases RAC1 and CDC42 (probably through the RHO guanine-nucleotide exchange factor (RHO-GEF) VAV2) and represses RHO by an unknown mechanism. Phosphatidylinositol 3-kinase (PI3K) is recruited to the membrane by intact E-cadherin adhesion junctions, where it generates phosphatidylinositol-(3,4,5)-triphosphate (PIP_3), resulting in the activation of the RHO-GEF TIAM1 and subsequently of RAC1 and CDC42. GTP-bound, activated RAC1 and CDC42 sequester the GTPase-activating protein IQGAP1, which in its free form would otherwise bind to β-catenin, thereby displacing α-catenin (α) from the CCC and disrupting the anchoring of the CCC to the cytoskeleton. Together, these activities affect the organization of the actin cytoskeleton, and possibly the migratory behaviour of tumour cells, as follows: activated CDC42 induces the formation of filopodia; activation of RAC1 results in the formation of lamellipodia; and activated RHO induces the formation of actin stress fibres. Cytoplasmic accumulation of p120-catenin can result in its translocation to the nucleus, where it associates with the transcription factor Kaiso and modulates gene expression. However, the functional implications of these changes in gene expression for tumour progression are not known. DSH, dishevelled; FRZ, frizzled; Ubi, ubiquitin.

and increased migratory and invasive-growth properties) indicates that loss of E-cadherin triggers active signals that initiate EMT. Based on the various interaction partners of E-cadherin and the connection of the CCC to the actin cytoskeleton, several potential signalling pathways are thought to have an active part in this process (FIG. 3).

Modulation of RTK signalling. Converse to the modulation of E-cadherin function by RTKs, functional adhesion junctions can also affect the activity of RTKs. For example, E-cadherin-mediated cell–cell adhesion has been shown to repress EGF-induced EGFR activation[31]. Contrary to these results, recent reports demonstrate that ligated E-cadherin can recruit EGFR and induce its ligand-independent activation, leading to the activation of signal-transduction cascades, including the phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways, and to tumour-cell survival[32,33]. Finally, E-cadherin-mediated cell adhesion induces the the activation and phosphorylation of the RTK EPHA2, resulting in the repression of cell–matrix adhesion and cell growth[34]. The involvement of these processes in the promotion or repression of tumour progression will be an important issue for future studies.

Activation of the WNT signalling pathway. As well as their crucial role in assembling the E-cadherin-mediated cell-adhesion complex, β-catenin and γ-catenin also have important functions in the canonical WNT SIGNALLING PATHWAY[35,36] (FIG. 3a). Non-sequestered, free β-catenin and γ-catenin are rapidly phosphorylated by glycogen synthase kinase 3β (GSK-3β) in the adenomatous polyposis coli (APC)–axin–GSK-3β complex and are subsequently degraded by the ubiquitin–proteasome pathway. If the tumour suppressor APC is non-functional, as in many colon cancer cells, or if GSK-3β activity is blocked by the activated WNT-signalling pathway, β-catenin accumulates at high levels in the cytoplasm. Subsequently, it translocates to the nucleus, where it binds to members of the TCF/LEF1 family of transcription factors and modulates the expression of their target genes, including c-*MYC*, cyclin D1, fibronectin, *MMP7*, *ID2*, *CD44*, *NrCAM*, axin-2 (conductin), *TCF1* and others, which are mostly genes implicated in cell proliferation and tumour progression.

This dual function of β-catenin has motivated several experiments to address whether the loss of E-cadherin function would subsequently lead to the activation of the WNT signalling pathway. In various cellular systems, it has been demonstrated that sequestration of β-catenin by E-cadherin can compete with the β-catenin/TCF-mediated transcriptional activity of the canonical WNT signalling pathway. The fact that E-cadherin does not completely deplete cytoplasmic β-catenin indicates that β-catenin exists in different functional pools[37–39]. Interestingly, in breast and prostate carcinoma cell lines, E-cadherin suppresses tumour-cell invasion by binding β-catenin without repressing β-catenin/TCF transcriptional activity, indicating that a novel, as yet unknown, additional function of β-catenin might be required for cellular invasiveness[40].

Signalling through RHO GTPases. Another signal that is elicited by the loss of E-cadherin function might involve changes in the organization of the cytoskeleton. Disassembly of the CCC and the concomitant loss of anchoring of the actin cytoskeleton to AJs apparently requires reorganization of the actin cytoskeleton. A key function in organizing the actin cytoskeleton is exerted by members of the RHO family of small GTPases; they control cytoskeletal organization and cell motility and, by doing so, they have been implicated in the regulation of tumour-cell proliferation and survival, in transformation and in tumour progression to malignancy[41].

Of the large family of RHO GTPases, RHOA, RAC1 and CDC42 are the prototype members that have been extensively studied. Similar to all other small GTPases, the activity of RHO proteins is upregulated by guanine nucleotide exchange factors (RHO-GEFs) and repressed by GTPase-activating proteins (RHO-GAPs). Following their activation, for example by growth-factor or hormone signalling or by integrin-mediated adhesion, downstream effector proteins — such as WAF1-activated kinases for RAC1 and CDC42- and RHO-associated coiled-coil-forming kinases (ROCKs) for RHOA — transduce signals to exert specific biological functions. CDC42 induces the formation of actin-rich spikes — filopodia — that are important in defining the directionality of movement; RAC1 induces the formation of actin-rich membrane ruffles — lamellipodia — at the leading edge of migrating cells; and RHOA regulates contractile forces to move the body and the tail of a migrating cell behind the leading edge and to induce the formation of actin stress fibres. Interestingly, RHO proteins can also modulate cell–cell adhesion by regulating cadherin activity[3] (FIG. 3b). But, how do RHO proteins and adhesion junctions communicate with each other?

E-cadherin, once engaged in cell–cell adhesion, suppresses RHO activity by activating p190 RHOGAP[42]. Notably, cadherin engagement induces tyrosine phosphorylation of p190 RHO-GAP, probably through SRC-family kinases, indicating that active signals are elicited by the formation of cell junctions. On the other hand, recent studies indicate that p120-catenin promotes cell migration by recruiting and activating RAC1 and CDC42, probably through the GEF VAV2, and by inhibiting RHOA[43,44] (FIG. 3b). Interestingly, only cytosolic p120-catenin is able to modulate small GTPase activity, whereas this function is abolished by the binding of p120-catenin to cadherins. Recent data indicate that p120-catenin, similar to β-catenin, is also able to translocate to the nucleus, where it binds to Kaiso, a member of the POZ family of zinc-finger transcription factors[45] (FIG. 3b). However, the functional involvement of such a transcriptional response in tumour progression has not been resolved.

WNT SIGNALLING PATHWAY
A developmental pathway of key importance for the patterning and specification of body axes in embryogenesis through activation of genes regulated by the TCF family of transcription factors. Deregulated WNT signalling has been implicated in various human tumours, most notably colon cancer, potentially by deregulating the balance between cell proliferation and differentiation.

Box 1 | The cadherin switch in development

Tumour progression is not the only context in which a change in the expression of various cadherin family members — a cadherin switch — has been observed. A conversion from epithelial (E)-cadherin to neuronal (N)-cadherin also occurs during epithelial–mesenchymal transition in embryonic development; for example, during gastrulation, when epiblast cells ingress the primitive streak[116,117], or when primordial germ cells migrate to populate the genital ridge[118]. However, in the latter instance, P-cadherin and E-cadherin are present during and after migration, whereas N-cadherin is expressed at post-migratory stages. Notably, the cadherin switch in epiblast cells can be recapitulated *in vitro* by treating the cells with hepatocyte growth factor, indicating that there is also a crucial role of signal transduction in cell adhesion during physiological processes[119]. In other instances, the cadherin switch leads instead to the loss of expression of mesenchymal cadherins during migration. In the chick embryo, for example, N-cadherin and cadherin-6B are expressed in the neural tube and in emerging neural-crest cells. During the delamination and migration of neural-crest cells from the neural tube, expression of N-cadherin and cadherin-6B is lost concomitantly with the upregulation of cadherin-7 (REF. 120). Sequential epithelialization of mesenchymal segmental plates to form somite and myotome compartments also requires N-cadherin, probably involving its cell–cell adhesion functions[121]. Consistent with this morphogenetic role, N-cadherin-deficient mouse embryos die of several developmental defects. Most importantly, the heart tube fails to develop normally and somites are small and irregular[122]. These and many other data indicate that during embryonic development, cadherins predominantly exert a cell-sorting and tissue-morphogenetic function based on their specific cell–cell adhesion capabilities. Whether cadherins are also involved in the stimulation of classical signal-transduction pathways in these processes remains to be investigated. First hints in this direction come from cultured primary neurons, where N-cadherin is able to stimulate fibroblast growth factor receptor signalling and, with it, neurite outgrowth (see Box 2).

In addition to interacting with RHO GTPases through p190 RHO-GAP and p120-catenin, E-cadherin can also communicate with these molecules through PI3K signalling. Ligation of E-cadherin molecules between two neighbouring cells recruits PI3K to the CCC, thereby generating phosphatidylinositol-(3,4,5)-triphosphate (PIP_3) at the plasma membrane. GEFs that contain PIP_3-binding pleckstrin-homology domains — such as TIAM1 — are then recruited to the membrane and activate RAC1 and possibly CDC42. These in turn induce actin assembly, probably through the ARP2/3 actin-nucleator complex[46] (FIG. 3b). The complexity of the regulation of GTPase activity is best illustrated by the various activities of TIAM1. It promotes the invasion and dissemination of T-cell lymphoma cells, an event that is thought to be mediated by RAC1 activation[47]. By contrast, it inhibits the migration and invasion of epithelial cells, when cultured on fibronectin and laminin, but promotes cell motility when cultured on collagen[48]. Consistent with such cell-context-dependent functions, *Tiam1*-knockout mice are partially resistant to phorbol-ester-induced carcinogenesis at earlier stages of tumour progression, but show an accelerated malignant conversion[49].

In addition to the effects of E-cadherin-mediated adhesion on the activity of RHO GTPases, cytoskeleton-associated signalling proteins also have an effect on the stability of the CCC. ASEF, a member of the DBL family of GEFs, decreases E-cadherin-mediated cell–cell adhesion and promotes the migration of MDCK (Madin–Darby canine kidney) cells[50]. By contrast, RAC1 and CDC42 can support E-cadherin function. IQGAP1, a downstream effector of RAC1 and CDC42, is known to negatively regulate E-cadherin-mediated cell–cell adhesion by interacting with β-catenin and displacing α-catenin from the CCC[51]. In their GTP-bound, activated form, RAC1 and CDC42 sequester IQGAP1 and prevent its binding to β-catenin, thereby stabilizing cadherin-mediated cell adhesion (FIG. 3b). Indeed, aberrant IQGAP1 expression and/or function has been observed during tumour progression, for example, in gastric cancer cells[52]. However, it remains to be determined whether IQGAP1-mediated disruption of E-cadherin function is a general process in tumour progression.

Unfortunately, the overall picture of RHO proteins and tumour progression is still quite murky. For example, changing the composition of the extracellular matrix will change the function of RAC1 from a pro-adhesive to an anti-adhesive molecule[48]. On the other hand, the functional roles of RHOC and the RHO effector ROCK in tumour metastasis have been clearly demonstrated in *in vivo* models of tumour-cell dissemination[53,54]. So, RHO family GTPases are certainly involved in many different aspects of the various stages of metastasis formation; however, their actual functional roles remain to be determined in more detail.

The cadherin switch

In several human cancer types, including melanoma, prostate and breast cancer, loss of E-cadherin function is accompanied by *de novo* expression of mesenchymal cadherins, such as N-cadherin and cadherin-11 (OB-cadherin)[55,56]. Cadherin-11 is expressed in invasive breast cancer and in breast cancer cell lines, and a carboxy-terminally truncated, alternatively spliced form of cadherin-11 can induce an invasive phenotype even in E-cadherin-positive breast cancer cell lines[57]. Upregulated expression of P-cadherin in breast cancers and of cadherin-6 in renal cell carcinoma also correlates with poor prognosis[58]. By contrast, T-cadherin (also known as H-cadherin) behaves more like E-cadherin: it is downregulated in basal and squamous-cell carcinomas of the skin, correlating with an invasive phenotype[59].

N-cadherin has been shown to promote cell motility and migration — an opposite effect to that of E-cadherin[60,61]. N-cadherin-induced tumour-cell invasion can even overcome E-cadherin-mediated

cell–cell adhesion[60,62]. Based on these observations, a novel theory has been put forward that a 'cadherin switch' similar to that involved in the delamination and migration of epithelial cells during embryonic development (BOX 1) also occurs during the transition from a benign to an invasive, malignant tumour phenotype[55,56].

One implication of the cadherin switch in tumour progression is that the change from E-cadherin to N-cadherin expression might provoke the tumour cell to move into different surroundings. Whereas E-cadherin is expressed by epithelial cells, mesenchymal cadherins are found in stromal cells, such as fibroblasts and myofibroblasts. It is conceivable that following loss of E-cadherin a tumour cell is no longer able to adhere to normal epithelial cells, whereas by upregulating N-cadherin expression tumour cells might be able to interact with stromal cells, thereby changing their location and invading the underlying stroma. Hence, N-cadherin (and, presumably, other mesenchymal cadherins) promotes a dynamic adhesion state in tumour cells, allowing not only the dissociation of single cells from the tumour mass but also their interaction with endothelial and stromal components[55,56,60].

In addition to this change in adhesion specificity, N-cadherin might provide the cells expressing it with an active, pro-migratory signal. In fact, expression of N-cadherin results in the downregulation of E-cadherin and correlates with increased invasion and motility. In BT-20 human breast epithelial cells, which express E-cadherin, N-cadherin could even induce motility and invasion without affecting E-cadherin levels, indicating that N-cadherin induces morphological changes that are independent of or dominant over E-cadherin function[62]. Moreover, MCF7 breast cancer cells transfected with N-cadherin show an increased metastatic potential following their injection into immunodeficient mice[60].

N-cadherin signalling

What is the signal that is elicited by N-cadherin and leads to increased motility? Similarly to neural CAM (NCAM, also known as CD56; see below), N-cadherin-mediated induction of FGFR signalling has been shown to occur in neurons, where it supports neurite outgrowth, an event that is closely related to cell migration and invasion[63] (BOX 2). Work in our laboratory has revealed a physical association between N-cadherin and different members of the FGFR family in various non-transformed and tumour-cell types[64]. Notably, in pancreatic β-cell tumour cell lines N-cadherin can physically interact with FGFR only in the presence of NCAM. A functional cooperation between N-cadherin and FGFR signalling has also been demonstrated in ovarian surface epithelial cells, resulting in cell survival[65], and in breast cancer cells, resulting in cell motility [66]. The association of N-cadherin with the FGFR is probably mediated by an interaction of the fourth extracellular cadherin domain of N-cadherin with the first two Ig-like domains of the FGFR[66]. It is thought that N-cadherin facilitates binding of FGF2 to the receptor, but prevents ligand-induced receptor internalization and downregulation. This leads to an increased cell-surface receptor level and sustained MAPK signalling, increased cellular motility and invasion, and the secretion of extracellular proteases, such as MMP9 (REF. 66). By contrast, in neurons, N-cadherin can stimulate responses through the FGFR in the absence of FGFs[67], indicating that N-cadherin can serve as a surrogate ligand for FGFR (BOX 2).

In addition to FGFR, N-cadherin also associates with c-MET, a receptor that is able to downregulate E-cadherin function (see above). This interaction enhances HGF-induced collagen invasion of retinal pigment epithelial cells[68], but the implications of this interaction for cancer progression are not clear. N-cadherin has also been implicated in survival signalling: on adhesion ligation, N-cadherin recruits PI3K to the adhesion complex, resulting in the activation of AKT (also known as protein kinase B) and increased cell survival[61,69]. Consistent with these results, U87MG glioblastoma cells depend on N-cadherin function to activate the PI3K–AKT pathway[70].

Another potential signalling pathway that might have a role in N-cadherin-mediated signalling involves the non-RTK FER, a FES proto-oncogene-related kinase that has been implicated in many different physiological processes, including cell adhesion, haematopoietic cell differentiation, blood clotting, retinal development and spermatogenesis. Experimental dissociation of FER from the juxtamembrane domain of N-cadherin causes the accumulation of FER in β_1-integrin complexes, resulting in a loss of both N-cadherin and β_1-integrin function[71]. The phosphotyrosine phosphatase PTP1B also shuttles between N-cadherin and β_1-integrins, and interference with PTP1B activity results in a loss of both N-cadherin and integrin function[72]. The presence of PTP1B in the cadherin complex is essential for dephosphorylation of β-catenin and, therefore, for N-cadherin function. Binding of PTP1B to N-cadherin requires its phosphorylation, and FER might be the kinase that phosphorylates PTP1B, targeting it to the cadherin complex. FER also phosphorylates p120-catenin, thereby modulating cadherin function. So, FER and PTP1B might modulate N-cadherin and integrin function, at least in part, by determining the phosphorylation status of components of the CCC. However, their role in tumour development remains unclear.

Signalling by other cadherins

Another example of a cadherin that interacts with an RTK is vascular endothelial (VE)-cadherin, a non-classical cadherin that is specifically expressed in endothelial cells, where it is critically involved in cell–cell adhesion and vascular integrity[73]. VE-cadherin associates with vascular endothelial growth factor receptor-2 (VEGF2) and modulates its signalling activities[74,75]. Interestingly, VE-cadherin has been shown to displace N-cadherin, which is also highly expressed in endothelial cells, from adhesion junctions[76]. However, it is not known whether this process changes the adhesive repertoire of endothelial cells or whether it modulates cadherin-mediated signalling; for example, by a shift from VEGFR to FGFR signalling. It is noteworthy that

VE-cadherin interacts with platelet–endothelial cell-adhesion molecule (PECAM, also known as CD31), a prototype member of the endothelial Ig-CAM family. Such interplay between the two adhesion systems is implicated in endothelial-tube formation[77], a crucial step in the angiogenic process. All these interactions certainly contribute to the functional role of VE-cadherin in the development and integrity of the vascular network. However, whether these processes are deregulated in tumour-associated angiogenesis and, therefore, contribute to tumour progression remains to be determined.

The role of NCAM in tumour progression

One of the best-studied members of the Ig-CAM family is NCAM. Its role in neurite outgrowth, axon guidance and long-term potentiation has been investigated in great detail[78]. However, NCAM is also expressed in

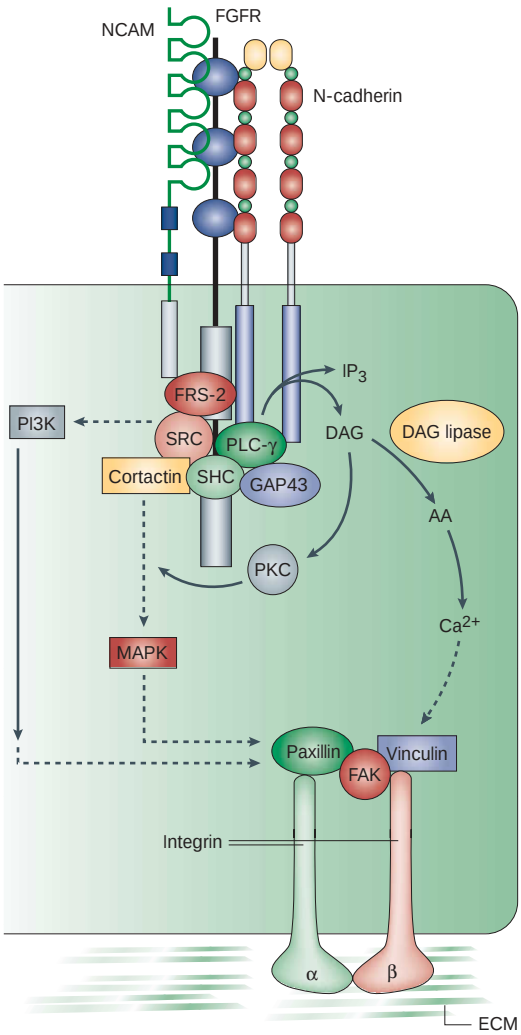
Box 2 | NCAM and N-cadherin signalling in neurons

Most of our knowledge about neural cell-adhesion molecule (NCAM) and neuronal (N)-cadherin function in cell adhesion and signal transduction comes from studies of neurite outgrowth in cultured neurons. Homophilic cell–cell adhesion in *trans* between neurons and NCAM-expressing feeder cells induces clustering of NCAM on neurons, which, in turn, results in the activation of the fibroblast growth factor receptor (FGFR)[63]. Notably, in neurons, both NCAM and N-cadherin seem to act as surrogate ligands for FGFR. The FGFR interaction domains have been identified and specific peptides resembling these domains are able to stimulate FGFR signalling [67,81]. FGFR activation results in the recruitment and activation of phospholipase Cγ (PLCγ). Subsequently, generation of diacylglycerol (DAG) and its conversion to arachidonic acid (AA) by DAG lipase results in an increase in calcium levels, thereby stimulating neurite outgrowth[63,123,124]. L1, another immunoglobulin-like CAM, exerts a similar function in the modulation of signal transduction and in the induction of neurite outgrowth[125].

The model that is shown in the figure is based on investigations of the induction of neurite outgrowth in primary neurons, PC12 pheochromocytoma cells and pancreatic β-cell tumour cell lines. NCAM, FGFR and N-cadherin might form a complex through their extracellular domains: NCAM interacts with FGFR, probably through its fibronectin type-III repeats, and N-cadherin with FGFR through its extracellular cadherin domain 4. Whether NCAM binds directly to N-cadherin is not known, but in pancreatic β-cell tumour cells the association of N-cadherin to FGFR is dependent on the presence of NCAM[64]. The signal-transduction pathways that are activated by the NCAM–FGFR4–N-cadherin complex ultimately lead to the activation of β_1-integrin-mediated cell–matrix adhesion and neurite outgrowth. The molecular links between NCAM-mediated signal transduction and integrin function remain to be elucidated; they are indicated in the figure by dashed arrows.

NCAM also associates with other signal-transduction molecules; for example, it interacts with FYN in the induction of neurite outgrowth, whereas L1 associates with SRC[126]. Differential localization of the various NCAM isoforms to membrane microdomains might thereby affect its interaction with signalling molecules: on palmitoylation, NCAM140 localizes to cholesterol-rich LIPID RAFTS and associates with FYN, whereas non-palmitoylated NCAM140 is found in non-raft membranes, where it associates with FGFR[127]. Both pathways are required for NCAM-induced neurite outgrowth.

NCAM is also a co-receptor for glia-derived neurotrophic factors (GDNFs). GDNFs usually interact with a receptor complex that is formed by the tyrosine kinase receptor RET and the glycosylphosphatidylinositol-linked co-receptor GFRα1 (REF. 128). In the absence of RET, NCAM associates with GFRα1, and binding of GDNFs induces the activation of FYN, resulting in Schwann-cell migration and neuronal differentiation[129].



ECM, extracellular matrix; FAK, focal adhesion kinase; FRS-2, FGFR substrate 2; GAP43, growth-activated protein 43; IP_3, inositol-trisphosphate; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C. Figure adapted with permission from REF. 64 © (2001) Nature Publishing Group.

LIPID RAFTS
Membrane microdomains that are distinguished from the rest of the plasma membrane by their lipid composition. They usually contain high levels of cholesterol (cholesterol-rich lipid rafts). Depending on their function or biochemical characteristics, such as lipid anchoring, proteins are differentially integrated into lipid rafts.

Box 3 | Distinct mechanisms of tumour-cell dissemination?

Metastatic spread is generally considered to be a process involving tumour cells that have acquired a highly invasive malignant phenotype. The loss of epithelial (E)-cadherin is a useful measurement for defining such a phenotype[130]. However, a significant proportion of various tumour types fails to show a correlation between the loss of E-cadherin and cancer progression and metastasis[131,132]. Moreover, metastases with relatively benign phenotypes have been detected in several studies[133,134]. In addition, the lymph-node metastases that develop in neural cell-adhesion molecule (*Ncam*)-deficient *Rip1Tag2* mice frequently have a relatively benign phenotype, as indicated by tumour-cell morphology and by the maintenance of E-cadherin expression[80].

What is the possible mechanism underlying the dissemination of E-cadherin-positive tumour cells? The impairment of cell–substrate adhesion and subsequent tissue disaggregation, as exemplified by *Ncam*-deficient *Rip1Tag2* tumours, results in the formation of HAEMORRHAGIC LACUNAE of blood and lymphatic vessels within the primary tumours[64] (see main text). This tumour disaggregation might provide a route for tumour-cell clusters that have detached from the tumour mass because of defective adhesion to leave the primary tumour site by means of the lymphatic drainage. 'Washed-out' cell clusters would then get trapped in the local lymph node, giving rise to tumour metastases. Such a process implies a 'passive' mechanism of tumour-cell dissemination, which does not require a transition towards an invasive, malignant phenotype[135]. Indeed, clusters of cancer cells at the adenoma stage have frequently been observed 'floating' in the haemorrhagic lacunae and lymphatic vessels of *Ncam*-deficient *Rip1Tag2* tumours[64]. Moreover, forced expression of the lymphangiogenic factor Vegfc during tumorigenesis in *Rip1Tag2* mice results in the formation of lymph-node metastases[136].

Of course, such a passive mechanism of tumour-cell dissemination does not rule out the classical process of metastasis, which requires cancer cells to actively invade the surrounding tissue, enter the circulation and colonize distant organs. In fact, the two metastatic pathways are likely to co-exist. Although a correlation between upregulated lymphangiogenesis and lymph-node metastasis has been established in many cancer types[137], future systematic analyses should assess the correlation between tumour-tissue disaggregation, lymphangiogenesis and benign metastases, in particular in regional lymph nodes. As well as contributing towards verifying the concept that passive tumour-cell dissemination can account for 'benign' metastasis[135], such analyses might help to explain why a significant number of patients with tumours (for example, those with breast cancer) show a favorable clinical course in spite of the presence of lymph-node metastases.

many other cell types, including epithelial cells of various organs, muscle cells and pancreatic β-cells. During the development of certain epithelial tumours, the expression pattern of NCAM undergoes marked changes. In colon carcinoma, pancreatic cancer and astrocytoma, NCAM expression is markedly downregulated and this loss of NCAM correlates with poor prognosis[79]. Moreover, in various cancer types, expression of NCAM shifts from the adult 120-kDa isoform to the embryonic 140-kDa and 180-kDa isoforms, together with a general downregulation of expression[79]. The biological significance of this change in NCAM expression and its role in tumour onset and/or progression are not understood, but it is certainly partly based on the differential polysialylation of the various NCAM isoforms, which has been shown to modulate the adhesive functions of NCAMs[78].

Using the *Rip1Tag2* mouse model, our laboratory has previously demonstrated that the loss of NCAM results in the induction of metastatic dissemination, predominantly to regional lymph nodes[80]. Notably, loss of NCAM resulted in a marked disaggregation phenotype in primary β-cell tumours, adenomas and carcinomas[64]. Consistent with this phenotype, tumour cell lines that were isolated from *Ncam*-deficient *Rip1Tag2* tumours revealed a defect in cell–matrix adhesion, although cell–cell adhesion was not affected by the loss of *Ncam*. This observation raises the possibility that tumour cells are able to form lymph-node metastases even in the presence of strong cell–cell adhesion. In contrast to the classical pathway of metastasis, which involves active tumour-cell migration, invasion and metastatic dissemination, following loss of cell–matrix adhesion, tumour cells might also be passively released from primary tumours and distributed — for example, by lymphatics to local draining lymph nodes. This hypothesis will have to be experimentally tested in the future (BOX 3).

Biochemical experiments to unravel the mechanism by which NCAM could affect cell–matrix adhesion have identified a signalling complex in which NCAM associates with FGFR4 and N-cadherin. As depicted in BOX 2 for NCAM-mediated signalling in neurons, this interaction stimulates all classical FGFR effector pathways, which, in turn, induce the activation of β_1-integrin and, therefore, cell–matrix adhesion[64]. Further studies are needed to elucidate whether the modulation of β_1-integrin activity by the NCAM–FGFR complex is required for the anti-metastatic function of NCAM *in vivo*. It is also unclear whether the loss of the ability of NCAM to modulate FGFR signalling and integrin function is causally implicated in those tumour types in which the downregulation of NCAM correlates with malignancy. Moreover, it remains to be elucidated whether there is a qualitative or quantitative difference between N-cadherin- and NCAM-induced FGFR signalling in neurons (BOX 2) or in tumour cells (see above). Furthermore, whether NCAM and N-cadherin act together to induce all the signalling outputs of FGFR or whether there are separable effects of the two during tumour progression will be a focus of future research.

HAEMORRHAGIC LACUNAE
Increased permeability or disruption of the endothelial lining of vascular or lymphatic vessels leads to leakage of blood or lymphatic fluid into the surrounding tissue, which, due to fluid pressure, results in the formation of fluid-filled lacunae.

Table 1 | Functional interactions of cadherins and Ig-CAMs with signalling molecules

CAM	Signalling molecule	Biological functions
E-cadherin	β-Catenin	If β-catenin is in a cadherin cell-adhesion complex, increase of cell–cell adhesion; if β-catenin is not sequestered, activation of WNT signalling and tumour-cell invasion
	c-MET	Disruption of intercellular adhesion
	IGF1R	Disruption of intercellular adhesion; enhancement of intercellular adhesion
	EGFR	Inhibition of ligand-dependent EGFR activation; ligand-independent activation of EGFR
	SRC	Loss of epithelial differentiation; invasiveness
	PI3K	Activation of AKT (also known as protein kinase B); cell survival
N-cadherin	FGFR1	Neurite outgrowth; cell migration and invasion; surivival of ovarian epithelial cells
	FGFR4	Neurite outgrowth; cell–matrix adhesion
	c-MET	Epithelial-cell invasion
	SRC	Inactivation of N-cadherin-mediated adhesion
	PI3K	Activation of AKT; cell survival
	FER	Crosstalk between N-cadherin and β_1-integrin
	PTP1B	Regulation of N-cadherin-mediated adhesion
VE-cadherin	VEGFR2	Endothelial-cell survival
	PECAM (also known as CD31)	Endothelial-tube formation
	DEP1	Modulation of VEGFR2 signalling
NCAM	FGFR1	Neurite outgrowth
	FGFR4	Neurite outgrowth; cell–matrix adhesion
	FYN	Activation of FAK
	PKCβ_2	Neurite outgrowth
	GFRα1	Schwann-cell migration; neuronal differentiation
L1	FGFR1	Neurite outgrowth
	Neuropilin	Modulation of semaphorin 3A signalling, axon guidance
	SRC	Neurite outgrowth
DCC	Robo	Axon guidance
	Adenosine A2b receptor	Neurite outgrowth
	DIP13β	Apoptosis

DCC, deleted in colorectal carcinoma; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; GFRα1, GDNF family receptor α1; IGF1R, insulin-like growth factor 1 receptor; NCAM, neural cell-adhesion molecule; PECAM, platelet–endothelial cell-adhesion molecule; PI3K, phosphatidylinositol 3-kinase; PKCβ_2, protein kinase C β_2; PTP1B, protein tyrosine phosphatase 1B; VEGFR2, vascular endothelial growth factor receptor 2.

Direct binding of NCAM to FGFR1 has recently been demonstrated by several biochemical experiments. The two FNIII domains of NCAM directly interact with Ig modules 2 and 3 of FGFR1, showing that the two molecules interact through their extracellular domains[81]. Peptides resembling NCAM FNIII domains are able to induce neurite outgrowth in neurons, indicating that the NCAM–FGFR interaction stimulates FGFR signalling even in the absence of FGFs, the *bona fide* ligands of FGFRs. Our studies with β-cell tumour cells also indicate that FGFs are not able to further stimulate FGFRs that are already activated by NCAM. Furthermore, whereas NCAM induces both neurite outgrowth and matrix adhesion through FGFR signalling, FGFs can replace NCAM only in the induction of matrix adhesion[64]. This is different from the interaction of N-cadherin with FGFR in breast cancer cells, which results in a sustained FGF-mediated stimulation of FGFR signalling[66] (see above).

In contrast to what has been discussed above, in neuroblastoma and certain neuroendocrine tumours, cancer progression correlates with the upregulation of NCAM[82–86]. However, the signalling properties of NCAM, its association with FGFR and N-cadherin, and its membrane localization have not been investigated in these tumour types. Interestingly, upregulation of NCAM in tumour cells is often accompanied by its extensive polysialylation, a post-translational modification that is frequently observed during development of the central nervous system[87]. So, the role of NCAM polysialylation in tumour progression and its effect on NCAM-mediated FGFR signalling still need to be investigated.

Roles of other Ig-CAMs in tumour progression

In addition to NCAM, several other Ig-CAMs show deregulated expression and/or function in various tumour types (TABLES 1,2). Accumulating experimental evidence indicates that at least some Ig-CAMs are involved in each stage of tumour progression.

Cell-adhesion molecules of the carcinoembryonic antigen (CEA) family have long been thought to have a role in tumorigenesis. The prototype member of the family, CEA, is upregulated in various epithelial tumours, such as colon, stomach, lung, pancreas and bladder tumours, and ovarian carcinoma[88]. However, despite CEA being widely used as a clinical marker for tumour progression, its functional role in tumour development is not known. In contrast to CEA, the expression of the related family member CEACAM1 (also known as biliary glycoprotein, C-CAM, or CD66a) is downregulated in various tumour types, including prostate, breast and colorectal tumours,

Table 2 | Involvement of Ig-CAMs in tumour progression

CAM	Tumour type	Changes in expression during tumour progression
NCAM	Pancreatic and colon cancer, astrocytoma Neuroblastoma, certain neuroendocrine tumours	Downregulated Upregulated
L1	Melanoma, breast and prostate cancer	Upregulated
DCC	Colorectal cancer, pancreatic cancer, neuroblastoma, various carcinomas	Downregulated
CEA	Various carcinomas	Upregulated
CEACAM1	Carcinoma of the prostate, breast, colon and endometrium	Downregulated
Mel-CAM	Melanoma, prostate cancer Breast cancer	Upregulated Downregulated
NrCAM	Pancreatic cancer Glioblastoma	Downregulated Upregulated

CAM, cell-adhesion molecule; CEA, carcinoembryonic antigen; DCC, deleted in colorectal cancer; Ig-CAM, immunoglobulin-like CAM; Mel-CAM, melanoma CAM; NCAM, neural CAM; NrCAM, neuronal CAM.

and endometrial carcinoma[89]. In addition, re-expression of CEACAM1 in cancer cell lines represses their tumorigenicity[90]. CEACAM1 also participates in several signal-transduction pathways, mainly due to its interactions with a wide range of signalling molecules, including protein tyrosine kinases and phosphatases[91]. Recent data indicate that CEACAM1 also modulates the angiogenic process. Interestingly, although CEACAM1 has been shown to induce neovascularization in certain experimental systems[92], it acts as an anti-angiogenic factor in prostate cancer[93].

The gene encoding the deleted in colorectal cancer (DCC) Ig-CAM was originally identified as a tumour suppressor, because of the high frequency of LOSS OF HETEROZYGOSITY (LOH) of this gene in colorectal cancer[94]. DCC has a central role in the development of the nervous system, where its functions are mainly regulated by its ligand, netrin, a laminin-like protein that acts as an axon guidance cue. The tumour-suppressive function of DCC has been questioned because of the fact that *Dcc*$^{+/-}$ mice do not show any increase in tumour incidence, even on crossing with the *APC*$^{MIN/+}$ MOUSE model of colon cancer[95]. Moreover, the *DCC* gene has been mapped to a locus that contains other genes with tumour-suppressive functions, such as *SMAD2* and *SMAD4* (also known as *DPC4*), raising the hypothesis that *DCC* mutations might not account for tumour progression due to mutations at this locus[96]. Nevertheless, the direct involvement of DCC in preventing malignancy is supported by several lines of experimental evidence. First, in various tumour types, *DCC* LOH occurs independently of *SMAD* mutations[97–99]; second, DCC has been reported to induce apoptosis[100], with obvious potential implications for limiting tumour growth; third, forced expression of DCC suppresses the tumorigenicity of various cancer cell lines[100]. However, the molecular details of how DCC exerts its tumour-suppressive functions remain to be elucidated and, therefore, the jury is still out on whether DCC deserves the title of a tumour suppressor.

L1 (also known as CD171) is an Ig-CAM that shows intriguing functional similarities to NCAM in neuronal differentiation[101] (BOX 2). The expression of L1 is also upregulated in certain tumour types, including breast cancer, prostate cancer and melanoma. The correlation between upregulated L1 expression and tumour progression seems to conflict with recent results showing that L1 suppresses the proliferation of transformed, but not normal, cell lines[102]. This apparent discrepancy might be resolved by considering that L1 might be involved in the regulation of metastatic dissemination rather than in tumour growth[103]. In particular, due to its ability to interact with integrins in a heterotypic manner, L1 has been proposed to favour the adhesion and transendothelial migration of melanoma cells, one crucial step in the metastatic dissemination of tumour cells[104].

Mel-CAM (also known as CD146, MCAM or MUC18) might be involved in melanoma pathogenesis. Indeed, the neoplastic transformation of melanocytes is accompanied by *de novo* expression of *Mel-CAM*, and the ectopic expression of *Mel-CAM* in melanoma cells induces tumour growth and metastasis[105]. Furthermore, antibody-mediated or genetic ablaton of Mel-CAM function suppresses the tumorigenic and metastatic phenotype of melanoma cells *in vivo*[106,107]. A correlation between *Mel-CAM* upregulation and tumour progression has also been reported in prostate cancer[108]. By contrast, the expression of *Mel-CAM* is lost during the progression of breast carcinoma, and experimental evidence indicates that *Mel-CAM* acts as a breast cancer suppressor[108]. Moreover, Mel-CAM promotes cell–cell interactions between melanoma cells and, similarly to L1, Mel-CAM favours the interaction of melanoma cells with endothelial cells, raising the possibility that Mel-CAM is implicated in the intra/extravasation of tumour cells. Ligated Mel-CAM recruits the SRC-related tyrosine kinase FYN, which then phosphorylates focal adhesion kinase[109]. In addition, Mel-CAM has been shown to reduce cell–matrix adhesion by downregulating the expression of β_1-integrins[110].

NrCAM, an Ig-CAM that is predominantly expressed in the brain, also shows a tumour-type-dependent behaviour. *NrCAM* is downregulated in highly malignant pancreatic cancers compared with differentiated tumour tissue[111], indicating an inhibitory role in tumour progression. Conversely, overexpression of *NrCAM* has been observed in glioblastomas, and NrCAM function is required for tumorigenicity of glioblastoma cell lines[112].

Future perspectives

As described above, E-cadherin, N-cadherin, NCAM and possibly other IgCAMs are able to associate with and modulate the activity of RTKs and other signalling molecules (TABLE 1). An increasing body of evidence now indicates that the functional interaction of many other cell-adhesion molecules — including integrins and the hyaluronan receptor CD44 — with RTKs and other signal transducers is a widespread phenomenon that might have implications for various physiological

LOSS OF HETEROZYGOSITY (LOH). In cells that carry a mutated allele of a tumour-suppressor gene, the gene becomes fully inactivated when the cell loses a large part of the chromosome carrying the wild-type allele. Regions with high frequency of LOH are believed to harbour tumour-suppressor genes.

APC$^{MIN/+}$ MOUSE
Mouse mode in which the adenomatous polyposis colon (*Apc*) tumour-suppressor gene carries a truncating mutation, resulting in a defective protein. These mice develop several benign polyps (adenomas) of the colon.

and pathological processes. The findings on integrins and CD44 are beyond the scope of this review, and have been summarized elsewhere[113–115].

Although many different examples of signalling mediated by cell-adhesion molecules have been reported, a general role in physiological and pathological processes still remains to be established. However, the fact that cell-adhesion molecules synergize with growth factors to stimulate RTK signal-transduction pathways, or are even able to induce these pathways in the absence of growth factors, adds an additional level of complexity to the functional investigation of signalling pathways in tumour development. Future experiments will be needed to assess whether such processes can be applied in general to different cancers types, whether any given RTK or other signalling molecule is functionally linked to one or several cell-adhesion molecules, or whether these molecular mechanisms are restricted to a few highly specific processes. Systematic analyses of the expression patterns of cell-adhesion proteins and of potential physical and/or functional interactions between those proteins and signalling molecules in different cancer types will be a first step towards this goal.

Many RTKs and other signal-transducing molecules have already been identified as attractive targets for the development of anticancer therapies. The fact that some of these signalling molecules regulate and, conversely, are regulated by cell adhesion strengthens the therapeutic strategies that are aimed at interfering with these signalling functions to prevent the dissemination of metastatic tumour cells. In summary, the functional implications of the crosstalk between CAMs and signalling molecules in the onset and/or progression of malignant disease will certainly be a key focus of future cancer research.

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Competing interests statement

The authors declare that they have no competing financial interests.

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