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The E-cadherin–catenin complex in tumour metastasis: structure, function and regulation

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Abstract

E-cadherin and the associated catenin complex have been recognised as performing a key role in cell adhesion. Loss of cell adhesion is seen as a key step in the cascade leading to tumour metastasis. The ability of both extra- and intracellular factors to regulate E-cadherin-mediated cell adhesion in physiological processes has provided insight into both the interactions of the E-cadherin–catenin complex, and possible mechanisms utilised by tumours in the process of metastasis. The interaction of the E-cadherin–catenin complex with various regulating factors, their effect on cell signalling pathways, and the relationship with the metastatic potential of tumours are reviewed. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: E-cadherin; Catenins; Cell adhesion; Cell signalling; Regulation; Tumour metastasis; Hypoxia; Growth factors; Epidermal growth factor receptor

1. Introduction

Since it was first recognised that tumours had the ability to invade adjacent tissues and spread to distant organs, extensive research has been performed, expanding the body of knowledge on this basic hallmark of malignancy. In recent years, with the advent of molecular techniques, crucial insights into the interplay of various factors at a molecular and genetic level have been gained. The resultant model shows metastasis to be a co-ordinated multi-step process encompassing the detachment of cells from the primary tumour to the development of a tumorigenic lesion at a distant site [1,2].

The process of metastasis appears to be regulated by a variety of gene products. These include cell–cell and cell–extracellular matrix receptors [3,4]; proteolytic enzymes that facilitate breakdown and invasion of the basement membrane, vascular channels and organs [5–7]; motility factors which allow migration through tissues [8,9]; receptors mediating organ-specific invasion [10]; growth factors necessary for the maintenance of the tumour microcolonies in the secondary organ [11]; and angiogenic factors that result in neovascularisation of the metastasis, allowing the supply of nutrients, removal of metabolites and haematogenous spread of metastatic cells [12,13]. Weakening of cell–cell adhesion is obviously imperative for tumour cells to metastasise. In recent years, several families of biochemically and genetically distinct cell adhesion molecules have been described. These include the cadherins, integrins, adhesion molecules belonging to the immunoglobulin superfamily, selectins and cell-determinant CD44.

The role of E-cadherin in metastasis has become topical in the past few years due to its apparent promise as a prognostic indicator, with loss or reduction of expression correlating with enhanced aggressiveness and dedifferentiation of many carcinomas [14–19]. Some tumours also display the ability to regulate E-cadherin expression during the process of metastasis, which raises questions about the role of the tumour microenvironment [20]. It has recently been hypothesised that hypoxia within tumours, resulting in tumour necrosis, causes downregulation of E-cadherin, and ultimately sets the metastatic cascade in motion [21]. In this paper, the interactions of the E-cadherin–catenin complex with various regulating factors, the consequences of the interactions on cell signalling pathways, and the relationship with the metastatic potential of tumours will be reviewed.

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2. The E-cadherin–catenin complex

The cadherin family consists of transmembrane glycoproteins responsible for calcium-dependent cell adhesion. The family is widespread in normal tissues but the individual members display pronounced tissue specificity. E-cadherin is one of the members of the family and is present within epithelial cells, where it tends to localise to specialised junctions of the *zonula adherens* type [22,23].

The human E-cadherin gene (*CDH1*) is situated on chromosome 16q22.1, within a large conserved-linkage group that includes loci for haptoglobin, chymotrypsinogen B, metallothionine-1, -2, tyrosine aminotransferase and lecithen: cholesterol acyltransferase [24–26]. E-cadherin forms from a 135 kDa precursor that undergoes cytoplasmic trimming of what will become the extracellular N-terminal end of the mature molecule. The extracellular N-terminal end is essential to the process of homophilic calcium-dependent cell–cell adhesion [27]. Following the trimming process, E-cadherin is routed towards the basolateral surface of the epithelial cell. The mature E-cadherin, weighing approximately 120 kDa, is composed of a highly conserved carboxy-terminal cytodomain, a single-pass transmembrane domain and an extracellular domain that consists of five tandemly repeated cadherin-motif subdomains, each harbouring two conserved regions representing the putative calcium binding sites (Fig. 1) [28,29]. The subdomains are numbered C1–C5 (where C1 is the most distant to the cell membrane) with the C1 subdomain containing a histidine–alanine–valine (HAV) sequence which is thought to be essential for the process of cell–cell adhesion [30,31].

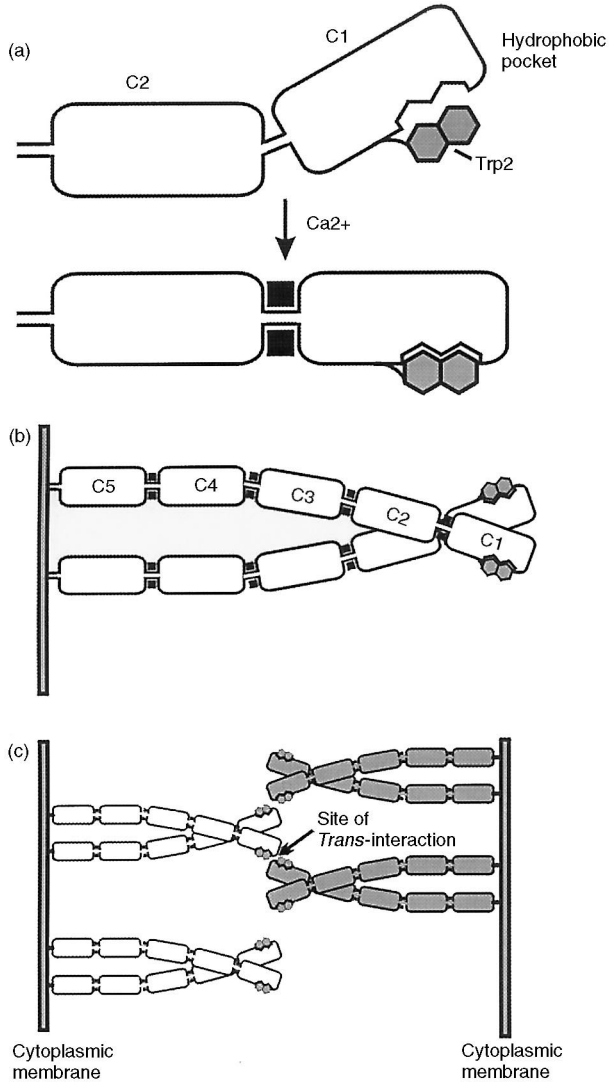

Fig. 1. The formation of E-cadherin bonds between cells. (a) Calcium binding induces a conformational change in the hydrophobic pocket partly formed by the histidine–alanine-valine (HAV) sequence. (b) The *cis*-interaction follows. (c) *Trans*-interaction results in adhesion between adjacent cells.

Recently, Pertz and colleagues have used a novel technique to generate crystals of a chimeric protein consisting of E-cadherin ectodomains fused to the pentamerising domain of cartilage oligomeric matrix protein [32]. The crystals have provided insight into the steric rearrangements underlying calcium dependency and the formation of *cis* and *trans* dimers of E-cadherin. There is a stepwise shift from a disordered cadherin structure via a rigid, rod-like, structure capable of *cis* dimerisation, to a *trans* dimer 'zipper' that forms between multiple X-shaped *cis* dimers of adjacent cells. It would appear that the affinity of calcium-binding sites for calcium varies among the subdomains, with the lowest affinity calcium-binding site being located at the junction of C1 and C2, which may inhibit *cis* dimerisation of C1 until the remainder of the ectodomain is correctly configured. The binding of calcium at the C1–C2 interface triggers a conformational change in the HAV structure allowing Trp-2 to move into a hydrophobic cavity with subsequent *trans* dimerisation [29,31,33].

In the past it has been assumed that the binding of the cadherin–catenin complex to the cytoskeleton was essential for the formation of strong cell–cell adhesion. Recent evidence has emerged which suggests that the clustering of *cis* dimers and the formation of lateral bonds between them can provide strong cell–cell adhesion [34–36]. The subsequent linkage to the cytoskeleton appears to stabilise the bonds moving them from a relatively weak state to a strong state over a period of approximately an hour [37–40]. It is thought that the formation of cadherin–catenin complexes following the formation of cell–cell attachment sets off a signalling pathway that results in attraction of E-cadherin. With time, freely diffusing E-cadherin becomes trapped by the immobilised cadherin–catenin complexes resulting in an increase of the local concentration of E-cadherin, which forms lateral bonds, strengthening cell–cell adhesion

[40,41]. This clustering would call into question the previous zipper hypothesis that described a continuous line of attachment between adherent cells [42].

The cytoplasmic domain of E-cadherin is the site of interaction with the catenin molecules that mediates its binding to the actin cytoskeleton. The cytoplasmic portion of E-cadherin contains a highly conserved region that is common to all members of the cadherin family [43]. There is a catenin recognition site within the cytoplasmic portion that forms the link to the cytoskeleton through its interaction with the catenin complex [44,45]. The two portions are connected by a single, 32 amino acid, hydrophobic membrane-spanning domain [43].

The catenin complex consists of α-catenin (102 kDa), β-catenin (92 kDa) and γ-catenin/plakoglobin (83 kDa) [46–48]. The human genes have been assigned for all three catenins, with α-catenin located on chromosome 5q31, β-catenin on chromosome 3p21, and γ-catenin/plakoglobin on chromosome 17q21 [49–51]. A fourth catenin-like molecule, $p120^{ctn}$ (also known as $p120^{cas}$), has recently been described and its gene localised to the long arm of chromosome 11q11 immediately adjacent to the centromere [52,53]. The protein has been shown to be a tyrosine kinase substrate for epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) receptors [54]. The catenins bind to E-caherin and each other in a specific manner. E-cadherin binds to either β-catenin or γ-catenin, while α-catenin also binds β-catenin or γ-catenin but not to E-cadherin [55–57]. The existence in the same cell of two distinct E-cadherin–catenin complexes results from the specific binding. One complex is composed of E-cadherin, α- and β-catenin, and the other of E-cadherin, α- and γ-catenin (Fig. 2) [57,58].

The E-cadherin–catenin complex begins to form during the passage of E-cadherin to the cell membrane. The first catenin to interact with E-cadherin is β-catenin [57,59]. The initial interaction is followed by binding of

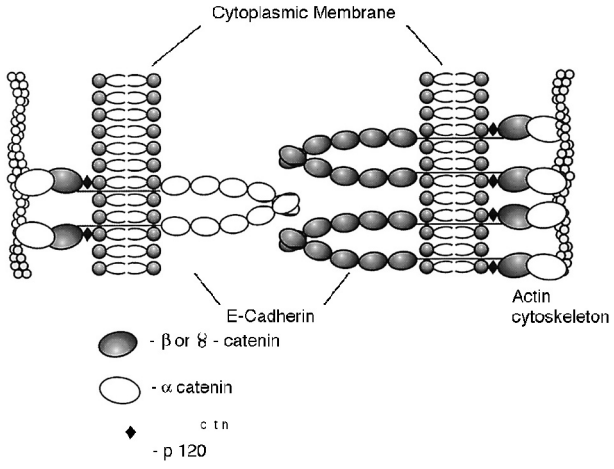

Fig. 2. The E-cadherin–catenin complex.

α-catenin to a short region close to the NH_2-terminal of β-catenin, which results in the formation of stable bonds between the complex and the actin cytoskeleton [60]. The binding domain responsible for the link between α-catenin and actin is located at the NH_2-terminal and is also responsible for the linkage of spectrin to the complex [61]. There has been some controversy surrounding the role of $p120^{ctn}$ with conflicting data concerning its role in the regulation and stabilisation of adhesion [34,62]. A recent study utilising a colon cell line (Colo 205) that has wild-type E-cadherin and catenins but that does not undergo aggregation, found that the post-translational modification of $p120^{ctn}$ (probably by phosphorylation) can regulate adhesion [63]. This implies a crucial role for $p120^{ctn}$ in the modulation of cadherin clustering and thus the stabilisation of adhesion.

3. Regulation of E-cadherin by interactions with the E-cadherin–catenin complex in non-transformed cells

Regulation of E-cadherin expression can occur in both physiological and pathological settings. Variations in E-cadherin expression have been noted during specific events in embryonic morphogenesis [64,65]. An example of such a process is during the development of the murine cochlea, where E-cadherin is downregulated on the lateral membranes of the reticular lamina, allowing the process of fluid space opening in the organ of Corti [66]. Embryonic processes have become important in providing models for the study of E-cadherin regulation. Another physiological process that involves regulation of cell adhesion is the reaction of epithelial cells in wound healing. The closure of the defect in an epithelial layer requires not only a reduction in cell adhesion but also stimulation of cell motility. The changes experienced by the epithelial cells would appear to be induced by the release of cytokines and other active substances following the injury. Of particular importance is the role played by EGF and its interaction with epidermal growth factor receptor (EGFR). Once the defect has been closed, cell adhesion is upregulated allowing the epithelial layer to regain its previous strength. Clearly lessons learnt from the study of the interactions of these active substances on the E-cadherin–catenin complex are vital in understanding the changes seen in tumours undergoing invasion and metastasis.

The importance of the E-cadherin–catenin system in the progression of tumours is well recognised. Reduction or loss of expression of E-cadherin has been documented in a significant proportion of tumours from varying organs, including colon [67,68], stomach [17,69,70], pancreas [71], oesophagus [72,73], liver [74], lung [73,75], bladder [76–78], prostate [79–81], breast [82–88], uterus [89], ovary [90], thyroid [91], skin and

oral carcinomas [92–96]. The degree of tumour differentiation appears to be related to the proportion of E-cadherin expression, with the poorly differentiated tumours more likely to show reduced E-cadherin expression that may be a result of downregulation or defects in the catenins [72,97–99].

As mentioned above, EGF acting through its receptor, EGFR, is an important regulator of E-cadherin. EGFR is actually a family of receptor tyrosine kinases comprising four members. The family includes ErbB1 (also referred to as EGFR), ErbB2 (also referred to as c-Neu), ErbB3 and ErbB4. The ligands for ErbB1 include EGF, transforming growth factor-α (TGF-α) and amphiregulin. These ligands are specific for ErbB1. Heparin-binding EGF-like growth factor (HB-EGF), betacellulin and epiregulin bind to both ErbB1 and ErbB4. The neuregulins and heregulins bind to ErbB3 and ErbB4 [100]. The activation and resultant signalling of EGFR after ligand binding is extremely complex and involves a variety of enzymes that are activated by tyrosine phosphorylation and adaptor molecules that form a link to downstream signalling pathways [101]. Treatment with epidermal growth factor appears to interfere with E-cadherin–catenin complex assembly and results in a more invasive phenotype *in vitro* [102]. The interference with complex assembly seems to be mediated by a mitogenic signal transmitted by EGFR through its tyrosine kinase resulting in tyrosine phosphorylation of β-catenin and E-cadherin itself [103]. The end result is dissociation of β-catenin from the E-cadherin–catenin complex and translocation of free β-catenin to the cytosolic pool. TGF-α has extensive homology with EGF, and produces most of the biological activities of EGF, due to binding with EGFR. Both TGF-α and EGF are released in response to epithelial injury and are thus important initiators in the process of wound healing. By their action through EGFR, they act to downregulate E-cadherin-mediated cell adhesion freeing the epithelial cells from their attachments and allowing them to migrate. This would appear to account for the downregulation of E-cadherin seen in epithelial cells adjacent to areas of ulceration in the gastrointestinal tract [104].

Other external factors that appear to exert a regulatory effect on the E-cadherin–catenin complex include autocrine motility factor (AMF), migration stimulation factor (MSF), scatter factor/hepatocyte growth factor (SF/HGF) and autotaxin [8,105,106]. The SF/HGF receptor, c-Met, is a transmembrane tyrosine kinase and proto-oncogene [107]. Binding of SF/HGF to c-Met appears to mediate mesenchymal/epithelial interactions that regulate cell growth, development, motility and morphogenesis [108,109]. HGF has been shown to induce scattering of epithelial cells, including Madin-Darby canine kidney (MDCK) cells, resulting in the cells growing as a dispersed culture rather than confluent islands [110–113]. The scattering implies a downregulation of E-cadherin–catenin cell–cell adhesion. Members of the small GTPase family have been implicated in the regulation of E-cadherin following stimulation of the c-Met receptor.

The small GTPase family consists of proteins whose function is dependent on the type of guanine nucleotide that is bound to them. There are two subfamilies: the Ras and the Rho subfamily. The Rho subfamily (consisting of Rho, Rac1, Cdc42 and several lesser-studied members) is of particular interest due to their ability to activate kinase cascades, induce gene transcription, and induce DNA synthesis [114]. Cdc42 and Rac1 appear to regulate cadherin-mediated cell adhesion, through their target IQGAP1, by inhibiting the interaction of IQGAP1 with β-catenin, leading to a stabilisation of the E-cadherin–catenin complex [115–117]. Cdc42 and Rac1 are found in two interconvertible forms: inactive GDP-bound and active GTP-bound forms [118]. Conversion from the inactive form to the active form is positively regulated by the GDP/GTP exchange factor (GEF) and negatively by the GDP-dissociation factor (GDI) [119,120]. The conversion from the active form to the inactive form is mediated by the GTPase-activating protein (GAP) [119]. When Cdc42 and Rac1 are in their active forms, they interact with IQGAP1, preventing it from interacting with β-catenin, thus stabilising the E-cadherin–catenin complex. In their inactive form cdc42 and Rac1 can not interact with IQGAP1, allowing it to interact with β-catenin, resulting in the dissolution of the E-cadherin-catenin complex with the dissociation of α-catenin, and thus loss of binding to the actin cytoskeleton [121]. The resultant cell adhesion is weak and unstable, being mediated merely by the interaction between E-cadherin structures described previously, and in all likelihood probably disintegrates fairly rapidly. It is hypothesised that the cell scattering induced by the action of HGF, through c-Met, may be mediated by this pathway, by promoting the actions of GAP and GDI, causing inactivation of Cdc42 and Rac1, allowing IQGAP1 to interact with β-catenin.

The small GTPase family is extremely active in a variety of signalling pathways, including the regulation of integrin-mediated signals that also have an effect on cell adhesion [122,123]. The regulation of these signals is, however, beyond the scope of this review, nevertheless they raise the distinct possibility of a globally interconnected pathway controlling cell adhesion. In a recent study, Rho, Rac and Cdc42 activation by tumour necrosis factor-alpha (TNF-α) resulted in re-organisation of the actin cytoskeleton and the formation of intercellular gaps, indicating reduced function of tight junctions [124]. A similar effect on endometrial epithelial cells by TNF-α had been noted previously and attributed to disassembly of actin filaments [125]. The evidence above appears to indicate a role for TNF-α in the regulation of cell adhesion opposite to that induced

by HGF. The reduction in cell adhesion associated with activated Rho, Rac and Cdc42 demonstrated by these studies is at variance with results in the literature and thus, the significance is unclear.

The publication of several studies reporting the ability of integrins to signal across the plasma membrane, resulting in local changes in cell adhesion and the cytoskeleton, fueled speculation that E-cadherin, itself, was involved in signalling [126]. EGF-induced signal transduction and its effects have been mentioned previously. Recently, an apparent intersection between signalling (WNT-wingless pathway) and adhesion (E-cadherin–catenin complex) has been demonstrated [127]. Wnt-1, which has been extensively studied in *Drosophila*, has been found to bind to a seven-transmembrane-domain receptor called Frizzled [128]. The association of Wnt-1 and the Frizzled protein families results in the activation of the Wnt-1 signalling pathway [129,130]. Since the identification of the Wnt-1 pathway it has been the subject of numerous studies to dissect its downstream interactions. The most proximal intracellular component is a protein known as Dishevelled (Dsh) [131]. Phosphorylation of Dsh to its active form is mediated by casein kinase II (CKII), but the stimulus for this process is not known [132]. Activated Dsh in turn inhibits glycogen synthase kinase-3β (GSK-3β), probably by phosphorylation of an amino-terminal serine residue of GSK-3β by protein kinase C (PKC) [133]. GSK-3β is a protein kinase that forms a complex with adenomatous polyposis coli (APC) protein, axin and free β-catenin within the cytosolic pool [134–136]. When in complex, GSK-3β acts in concert with axin to phosphorylate β-catenin and APC [134,137]. The phosphorylated β-catenin undergoes ubiquitination followed by degradation mediated by proteosome [138]. It can thus be seen that inhibition of GSK-3β will have the effect of reducing β-catenin degradation, therefore increasing the pool of free β-catenin.

The increased pool of β-catenin, mediated by the inactivation of GSK-3β will obviously have an effect on cell adhesion with the β-catenin in the cytosolic pool either linking with E-cadherin or acting in the Wnt-1 pathway. If the stimulus that activated the Wnt-1 pathway also resulted in the activation of EGFR, then it is clear that movement back to the cell adhesion complex would not be possible. It follows that free β-catenin may heterodimerise with members of the leucocyte enhancer factor/T-cell factor (LEF/TCF) family allowing translocation to the nucleus, where the complex of β-catenin and LEF/TCF induces DNA bending and transcription of Wnt-responsive genes [139–142]. The nature of the target genes has been largely unknown until recently. A study by He and colleagues discovered the presence of LEF binding sequences in the promoter region of *c-MYC* [143]. Shtutman and associates have recently described the presence of LEF binding sequences in the promoter region of the *cyclin D1* gene [144]. Cyclin D1 is a major regulator of cell proliferation through its shift of cells into the proliferative stage of the cell cycle. The same study has demonstrated that transcriptional activation of the *cyclin D1* gene was inhibited by enhancing the degradation of β-catenin with wild-type (wt) APC and axin or by binding to the cytoplasmic tail of E-cadherin [144]. LEF binding sites have also been found contained within the promoter region of the E-cadherin gene, *CDH1*, and it has been proposed that binding of the complex of β-catenin and LEF/TCF downregulates the expression of *CDH1* [145].

From the mechanisms described above, it can be appreciated that the regulation of cell adhesion mediated by E-cadherin is extremely complex. Most of the factors appear to exert their effects through actions on β-catenin, either stabilising its bond with E-cadherin and α-catenin, promoting cell–cell adhesion, or dissolving its bonds resulting in a break up of the E-cadherin–catenin complex, causing downregulation of cell–cell adhesion. The dissection of these pathways is intricate and one may become overwhelmed with data. Yet, if the process is viewed on a larger scale the interactions become clearer (Fig. 3). Combined stimulation of EGFR and c-Met receptors has the effect of translocating β-catenin into the cytosolic pool where it can be degraded. If the Wnt1 pathway is activated at the same time as the other two pathways, the degradation of β-catenin is inhibited and it is translocated to the nucleus to combine with LEF/TCF, causing transcription of the *cyclin D1* gene and downregulation of the *CDH1* gene. The net result of all these interactions is a reduction in E-cadherin-mediated cell adhesion and a proliferation of cells. When a process such as wound healing is considered, a proliferating cell population with reduced cell adhesion would be perfect to replace a destroyed or traumatised epithelium.

Obviously this process would require controlling mechanisms. Contact inhibition is a well-known and accepted phenomenon, but despite this the details of its mechanism are poorly understood. Recently, study of cyclin-dependent kinase inhibitors (CKIs) has revealed that $p27^{Kip1}$ is involved in contact inhibition [146,147]. It has been demonstrated that as the density in cell cultures increases, so does the mRNA expression of p15, p16 and p27 and cyclin-dependent kinase-2 activity is markedly reduced eventually resulting in density-mediated growth arrest [147]. Transforming growth factor-β (TGF-β) can cause a similar growth arrest, probably mediated by CKIs [148]. The likelihood of contact inhibition being mediated by homophilic E-cadherin binding is high although no data are available at present. If this is so, then it would suggest the presence of an, as yet, undissected pathway, involving stimulation of CKIs, reversal of cyclin D1-mediated proliferation and an increase in transcription of the *CDH1* gene. An upreg-

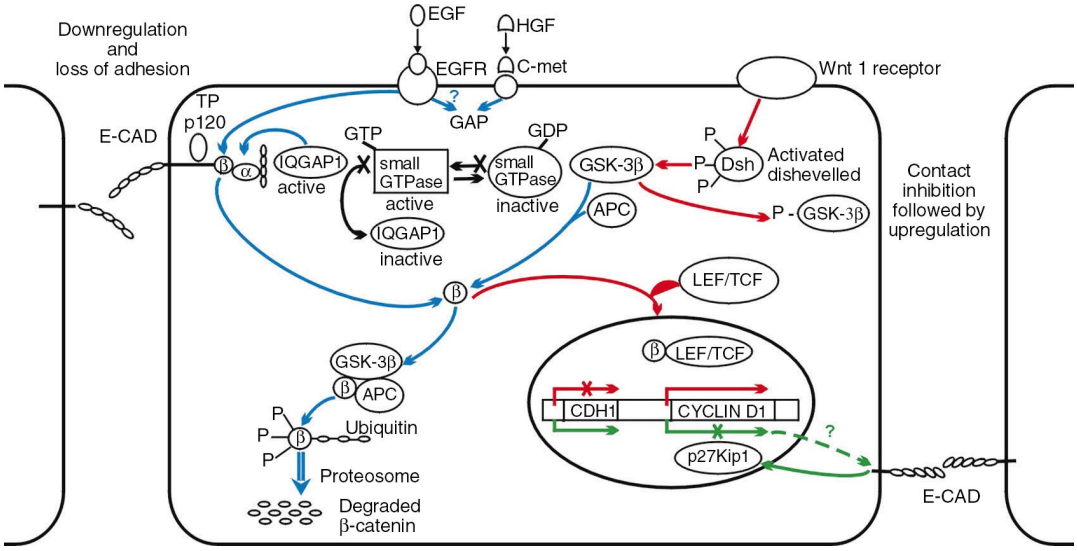

Fig. 3. A model illustrating the interaction between E-cadherin-mediated cell adhesion and cell signalling resulting in downregulation of E-cadherin. Reversal by way of contact inhibition is poorly understood. E-cad, E-cadherin; α, α-catenin; β, β-catenin; TP, tyrosine phosphorylation; P, phosphorylation; EGF, epidermal growth factor; HGF, hepatocyte growth factor; EGFR, epidermal growth factor receptor; GAP, GTPase-activating protein; GSK-3β, glycogen synthase kinase-3β; APC, adenomatous polyposis coli protein; Dsh, dishevelled protein; LEF/TCF, leucocyte enhancer factor/T-cell factor.

ulation of E-cadherin mediated cell–cell regulation would require reversal of the pathways enumerated previously.

As previously mentioned, the role of $p120^{ctn}$ in cell adhesion is the subject of much controversy and debate at the moment. Various studies have given rise to ambiguous results regarding a consistent role for $p120^{ctn}$ in the regulation of cell adhesion. It has been proposed that $p120^{ctn}$ binding is permissive for cadherin function, while others argue that the presence of a $p120^{ctn}$-binding domain in the absence of the catenin-binding domain inhibits cadherin-mediated aggregation [34,62]. It is possible that $p120^{ctn}$ interactions are complex, with the function depending on the binding of a particular isoform and various post-transcriptional or translational modifications [149,150].

The Src family of protein receptor kinases has been linked to mitogenic pathways activated by EGFR. Src appears to function by increasing the number of cells in a given population that are able to respond to EGF stimulation [151]. A link between Src overexpression and increased basal tyrosine phosphorylation of p190RhoGAP has been identified suggesting a role in the regulation of the small GTPases [152]. In view of the interactions of Src with EGFR and GAP, the possibility of a link between EGFR and the small GTPases exists, although there is no direct evidence at present to support it.

4. Regulation of E-cadherin in malignant cells

The E-cadherin–catenin complex, that mediates cell adhesion, is dependent on numerous interactions that have been highlighted above. Thus, it should be obvious that cell adhesion is not solely dependent on the structural and functional integrity of the E-cadherin molecule but also that of the associated catenins and other molecules that mediate its binding to the cytoskeleton. Reduction in cell adhesion is of major importance in tumour metastasis and appears to be achieved by a variety of mechanisms affecting the E-cadherin–catenin complex. These include reduction or loss of E-cadherin expression, mutation or reduced transcription of the genes of the constituent molecules, redistribution of E-cadherin to different sites within the cell, shedding of E-cadherin, and competition for binding sites from other proteins [153].

The processes that result in downregulation of E-cadherin-mediated cell adhesion in non-transformed cells have been dealt with previously. From the resultant model (Fig. 3) it can be seen that cell adhesion may be down regulated by external stimuli activating the relevant pathways or by mutation to one of the components of the pathways. Stimulation of EGFR on malignant cells by EGF or TGF-α will result in E-cadherin downregulation by the mechanisms previously described. The EGF or TGF-α could either be derived from the surrounding tumour microenvironment, due to tumour hypoxia and necrosis, or be excreted from the tumour cells themselves, resulting in an autocrine effect.

The expression of EGFR has been associated with a more invasive phenotype, lymph node metastases and worse prognosis in a variety of malignancies, including small-cell carcinoma of the lung, squamous carcinoma of the larynx, papillary carcinoma of the thyroid, invasive duct carcinoma of the breast, and many others

[154–158]. Increased expression of TGF-α, EGF and insulin-like growth factor 1 (IGF-1) has been found in a significant proportion of breast carcinomas [158–160]. Breast stromal cell cultures derived from human breast cancer lines are able to secrete an EGF-like substance that is thought to be a result of stimulation by the adjacent cancer cells [161]. These results would seem to indicate that reaction to varying concentrations of stimulatory factors, such as EGF and TNF-α, within the various microenvironments of a tumour, as well as the presence of EGFR, affect the degree of cell adhesion allowing the carcinoma to transform into an invasive tumour.

The *APC* gene is located on chromosome 5q and is mutated in familial adenomatous polyposis (FAP) and in the majority of sporadic colorectal carcinomas [162,163]. The mutations described in FAP usually result in truncations causing loss of the C-terminal region of APC that contains the microtubule site [164]. The effect of this inability to bind and stabilise microtubules may compromise the migration of the gut crypt epithelial cell to the luminal aspect [165]. In addition, the ability of mutated (mt) APC to constitute a complex with and degrade free β-catenin, in the presence of axin and GSK-3β, is compromised, causing an increase in the cytosolic free β-catenin. In transformed cultured epithelial cells, the large pool of free β-catenin is reduced on addition of wt APC, confirming the crucial role it plays in β-catenin degradation [166,167]. The effect of an increase in free β-catenin would be not only an increase in E-cadherin-mediated adhesion but also increased proliferation due to the action of the β-catenin and LEF/TCF complex. The inability of the crypt epithelial cells to migrate would expose them to toxins within the lumen of the gut for a prolonged period. This together with the increased cellular proliferation may lead to an accumulation of additional mutations, transforming the adenoma into a carcinoma [165,168].

Mutation of the genes of the constituent proteins forming the E-cadherin–catenin complex may result in structural or functional aberrations that result in a reduction in cell adhesion. Mutations of the *CDH1* gene appear to be infrequent events. In-frame skipping of exons 8 or 9 and deletion of exon 10 have been demonstrated with diffuse-type gastric cancer [169]. Point mutations in exons 7 (invasive breast carcinoma), 12 and 13 (endometrial carcinoma) and 16 (ovarian carcinoma) have also been demonstrated that mostly affects the extracellular domain of E-cadherin [170,171]. Probably the best known tumours exhibiting mutations of *CDH1* are invasive lobular carcinoma of the breast and diffuse gastric cancer, with approximately 50% of these tumours being affected [172–174]. A germ line mutation of *CDH1* has been detected in various families, that results in the development of poorly differentiated diffuse-type adenocarcinomas with signet-ring cells [175,176]. The term hereditary diffuse gastric cancer (HDGC) syndrome has been coined to describe affected patients [177]. The reason why gastric cancer predominates in the affected families is not clear.

The role of an increased free pool of β-catenin on gene transcription has been discussed above. Recently, it has been demonstrated that overexpression of the integrin-linked protein kinase $p59^{ILK}$ stimulates the action of the β-catenin and LEF/TCF complex causing a downregulation of E-cadherin expression through the reduction in *CDH1* transcription [178]. The result is a reduction in cell adhesion and acquisition of a more invasive phenotype and indicates a link between integrin signalling and the regulation of cadherin-mediated cell adhesion. Deletions of the α-catenin gene, resulting in a mutated α-catenin that does not bind E-cadherin, have been identified in lung, colon and prostate carcinoma cells [79,179–181]. The β-catenin gene has been found to be deleted in a human gastric carcinoma cell line [97,99].

Redistribution of E-cadherin expression has been noted in some cancers, with the staining being variable or spotty in distribution, or located in abnormal sites along the membrane [73,75]. Cytoplasmic (as opposed to membranous) expression has been noted in thyroid, breast and some squamous carcinomas [87,90,182]. A recent study found that a reduction in membranous β-catenin and increased cytoplasmic E-cadherin expression in gastric cancer was associated with poor survival [183]. It was noted that some of the tumours studied showed cytoplasmic staining, whereas others had nuclear staining. This distribution may give a clue as to the driving force behind the shift, with cytoplasmic staining probably representing an increase in the cytosolic pool of free β-catenin, while the nuclear staining may represent β-catenin in complex with LEF/TCF. Cytoplasmic accumulation of α-catenin in laryngeal squamous carcinomas appears to be associated with dedifferentiation and aggressive behaviour [184].

Shedding of E-cadherin from the cell surface, with resultant excretion of soluble E-cadherin in the urine and peritoneal lavage specimens has been reported [185–187]. The primary tumours in these cases have been noted to show reduced E-cadherin expression. Bladder cancers have also been shown to be associated with shedding of the molecule into the urine [188].

5. The role of hypoxia in E-cadherin regulation and metastasis

It is clear that factors external to the cell have a major influence on cell adhesion. The milieu in which the transformed cells of malignant tumours exist is bound to be very different from that found in normal tissues. By their very nature, malignant tumours act to create an environment that aids their transformation and progression. Growth by cellular proliferation is the main

function of malignant tumours and it occurs at the expense of all other cellular activities. The disorganised architecture, that is characteristic of malignancy, provides inefficient mechanisms for the removal of waste products of cellular metabolism resulting in a relatively toxic environment in which the transformed cells continue to proliferate. The search for factors that affect the progression and behaviour of tumours has led to the investigation of the effect of the microenvironment on particular tumours.

A specific microenvironmental factor occurring in malignant tumours that is receiving attention is that of hypoxia. Oxygen deprivation would seem to be a factor that is present in almost all malignant tumours. With progressive and rapid growth of the tumour population, the blood supply is outstripped resulting in cellular ischaemia and eventually the tumour necrosis that is invariably seen in malignant tumours. The overall effect of hypoxia on tumours appears to adversely affect the prognosis of the patients. Well-documented examples of this include carcinomas of the head and neck and cervical carcinomas [189,190] The presence of hypoxia within these tumours has been associated with increased invasiveness and a propensity to metastasise. Soft tissue sarcomas with reduced oxygen levels have been shown to have a worse prognosis compared with those with higher oxygen tensions [191]. Other instances where tumours exposed to hypoxia assume a more aggressive phenotype are those tumours that have been subjected to subcurative radiotherapy [192,193]. The hypoxia in such a setting appears to be mediated by the vascular changes seen in response to radiotherapy.

It has recently been hypothesised that the progression of malignant tumours is mediated by hypoxia and tumour necrosis, that set in motion a chain of events leading to the acquisition of a metastatic phenotype (Fig. 4) [21]. To summarise, a malignant tumour is present that undergoes uncontrolled growth, eventually outstripping its blood supply, resulting in hypoxia and starvation due to lack of nutrients. The resultant necrosis releases active substances, including cytokines, peptide growth factors and cytotoxic factors such as nitric oxide (NO). The result of this is a population of cells that are exposed to sublethal ischaemia, which has the effect of reducing cell adhesion, increasing DNA mutations and stimulating angiogenesis. With the ingrowth of new vessels and reoxygenation of the affected cells, the resultant clone assumes a more aggressive behaviour due to the acquisition of a large number of genomic mutations imparting a metastatic phenotype. The transformed, poorly adhered cells that show reduced E-cadherin expression now have the ideal opportunity to invade adjacent tissue and the newly formed delicate vessels provided by the process of angiogenesis, using all the well-described mechanisms of the metastatic cascade that are beyond the scope of this review. It is thus clear

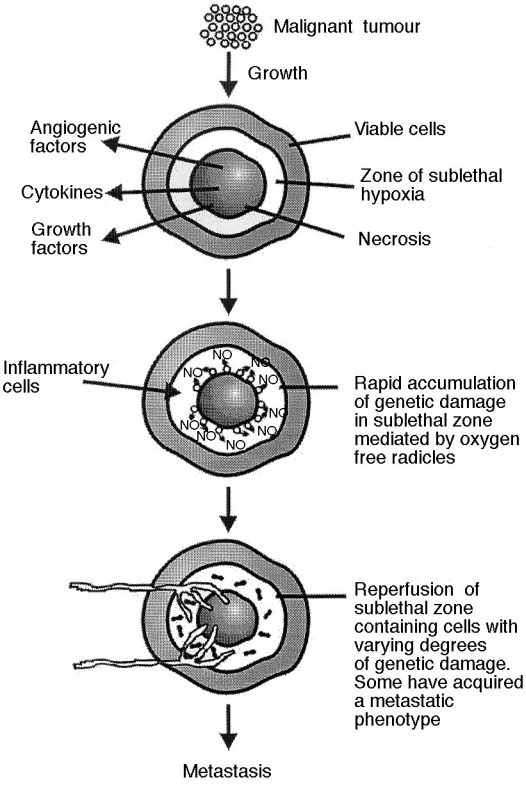

Fig. 4. A diagrammatic summary of the 'hypoxia' hypothesis.

from the hypothesis, that although the development of hypoxia within the tumour is essential for the initiation and promotion of the metastatic cascade, it is the resultant angiogenic response that allows the tumour to reach its full potential and metastasise.

There are data in the literature to support the hypothesis. Peptide growth factors, such as EGF and TNF-α, are released in response to hypoxia and will downregulate E-cadherin as previously described [194,195]. EGF appears to have a protective effect on cells exposed to hypoxia [196]. The demonstration of nitric oxide synthetase (NOS) within high grade tumours, suggests that the resultant formation of NO, that acts as a free radical, may provide a positive growth signal within the hypoxic tumour environment, resulting in increased growth rate, vascular density and invasiveness [197]. Other active substances released by hypoxia and tumour necrosis, that act to increase invasion and metastasis, include urokinase-type plasminogen activator and its receptor, interleukin-8 and hypoxia-inducible factor 1 alpha [198–202]. Recently, a new anticancer drug, tirapazamine, has been developed, that appears to be activated under hypoxic conditions and is highly toxic to hypoxic but not aerobic cells [203,204]. Clearly, tirapazamine may prove useful in chemotherapeutic regimens, because if treatment is instituted prior to metastasis, it may prove lethal to those tumour cells that have been transformed to a metastatic phenotype by the hypoxic environment, thereby preventing metastasis.

6. Conclusion

It has been demonstrated that the control of E-cadherin-mediated cell adhesion in benign and malignant epithelial cells is complex and relies on interactions between various external factors and intracellular signalling pathways. The loss or downregulation of E-cadherin is a key event in the process of tumour invasion and metastasis. As understanding of the interactions involving components of the E-cadherin system increases, hopefully so will the ability to predict and combat these events, leading to a reduction in tumour metastasis. It is, however, important not to lose sight of the fact that the process of tumour invasion and metastasis is complex and interwoven, and targeting a single part of this process may not successfully halt it. Clearly, research to date has merely revealed the tip of the process, and much work still lies ahead, particularly in relatively neglected areas, such as the role that hypoxia exerts on cell adhesion, tumour progression and metastasis.

7. The outstanding questions

- What is the true role of $p120^{ctn}$ in cell adhesion?
- What is the pathway through which E-cadherin stimulates contact adhesion and does it have a role in tumour progression?
- Does EGFR stimulation have an effect on the small GTPases, possibly through an interaction with GAP?
- Can E-cadherin downregulation be blocked?
- Does hypoxia initiate the metastatic cascade?
- Does tirapazamine have a role to play in the prevention of metastasis?

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