Review

E-cadherin–catenin cell–cell adhesion complex and human cancer

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Background: **The E-cadherin–catenin complex plays a crucial role in epithelial cell–cell adhesion and in the maintenance of tissue architecture. Perturbation in the expression or function of this complex results in loss of intercellular adhesion, with possible consequent cell transformation and tumour progression. Recently, much progress has been made in understanding the interaction between the different components of this protein complex and how this cell–cell adhesion complex is modulated in cancer cells.**

Methods: **This is an update of the role of the E-cadherin–catenin complex in human cancers. It emphasizes new features and the possible role of the complex in clinical practice, discussed in the light of 165 references obtained from the Medline database from 1995 to 1999.**

Results: **More evidence is now appearing to suggest that disturbance in protein–protein interaction in the E-cadherin–catenin adhesion complex is one of the main events in the early and late steps of cancer development. An inverse correlation is found between expression of the E-cadherin–catenin complex and the invasive behaviour of tumour cells. Therefore, E-cadherin–catenin may become a significant prognostic marker for tumour behaviour. Besides its role in establishing tight cell–cell adhesion, β-catenin plays a major role in cell signalling and promotion of neoplastic growth. This suggests its dual role as a tumour suppressor and as an oncogene in human cancers.**

Conclusion: **Recent developments show that the E-cadherin–catenin complex is more than a 'sticky molecular complex'. Further studies may yield greater insight into the early molecular interactions critical to the initiation and progression of tumours. This should aid the development of novel strategies for both prevention and treatment of cancer.**

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Introduction

It has long been known that cell–cell adhesion is generally reduced in human cancers. Reduced cell–cell adhesiveness is associated with loss of contact inhibition of proliferation, thereby allowing escape from growth control signals[1]. Invasion and metastases, the most life-threatening properties of malignant tumours, are considered to be later, but critically important, carcinogenic steps. These steps consist of sequential changes in host–tumour interaction. The suppression of cell–cell adhesiveness may trigger the release of cancer cells from the primary cancer nests and confer invasive properties on a tumour. In order for a metastatic nodule to form, cancer cells must detach from the primary site, invade through the surrounding host tissue, enter the circulation, lodge in a distant vascular bed, extravasate into the target organ, and proliferate[2]. Reduced cell–cell adhesiveness is considered indispensable for both the early and the late carcinogenic steps. In recent years, there has been increasing interest in a large family of transmembrane glycoproteins, called cadherins, which are the prime mediators of calcium-dependent cell–cell adhesion in normal cells[3]. There is increasing evidence that modulation of this complex by different mechanisms is an important step in the initiation and progression of human cancers.

Cadherins

The cadherins have been divided up into more than 10 subclasses, depending on their tissue distribution; these

include E- (epithelial), N- (neuronal) and P- (placental) cadherins[4,5]. E-cadherin (120 kDa; chromosome 16q), otherwise known as uvomorulin, L-CAM, cell-CAM 120/80 or Arc-1, is a classical cadherin and forms the key functional component of adherens junctions between epithelial cells[6]. Normal E-cadherin expression and function are essential for the induction and maintenance of polarized and differentiated epithelia during embryonic development[3]. The critical importance of E-cadherin in normal development and tissue function is demonstrated by the lethality of E-cadherin knockout mice at an early stage in embryogenesis[7]. The binding of cadherins is homotypic in nature, i.e. they bind only to identical molecules on adjacent cell surfaces. The contribution of cadherins to intercellular binding is achieved through the formation of cell surface multimolecular structures with a 'zipper' confirmation[8].

Catenins

Cadherin binding requires a complex series of interactions between cadherins and cytoplasmic molecules. E-cadherin is bound via series of undercoat proteins, the catenins, to the actin cytoskeleton[9] (*Fig. 1*). This linkage between transmembranous cadherins and actin filaments of the cytoskeleton is necessary to form strong cell–cell adhesion[10]. Deletion of the intracellular catenin-binding domain of E-cadherin or alteration of the functionally active catenins results in loss of the ability of E-cadherin to establish cell–cell adhesion, even if the extracellular binding domain is intact[10]. The catenin family comprises α- (102 kDa, chromosome 5q21–22)[11], β- (92 kDa; chromosome 3p22)[12] and γ- (plakoglobin; 83 kDa; chromosome 11q11)[13] catenins, with β- and γ-catenin sharing the greatest homology. β-Catenin and γ-catenin bind directly to the cytoplasmic tail of E-cadherin in a mutually exclusive manner; α-catenin then links the bound β- or γ-catenin to the actin microfilament network of the cytoskeleton. Recently, another catenin-like molecule, $p120^{ctn}$, has been identified in association with E-cadherin at the cell–cell junctions, although this complex does not appear to form a link with the actin cytoskeleton[14]. Originally identified as one of the several substrates of tyrosine kinase pp60src, $p120^{ctn}$ also associates with β-catenin and E-cadherin[15]. Unlike the other catenins, it has four isoforms, which appear to be expressed differentially in a variety of cell types[14]. Recently, it has been shown that $p120^{ctn}$ acts as an inhibitory regulator of cadherin function in colon carcinomas[16].

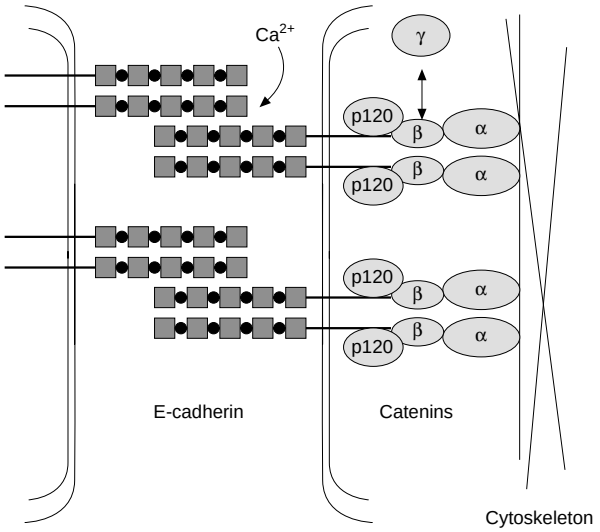

Fig. 1 Schematic representation of cadherin-mediated cell–cell adhesion in epithelial cells. Intercellular binding is achieved through the formation of E-cadherin dimers that interdigitate to form a continuous linear 'zipper' structure. The interaction is dependent on extracellular calcium levels. The cytoplasmic domain of E-cadherin is complexed with either β- or γ-catenin. α-Catenin links the complex to the actin cytoskeleton of the cell

E-cadherin–catenin complex in tumour development

An intact E-cadherin–catenin complex is required for maintenance of normal intercellular adhesion. In the light of this, several groups have proposed that in carcinomas E-cadherin functions as an invasion suppressor molecule such that its loss permits or enhances the invasion of adjacent normal tissues. There are many data to support this hypothesis. Immunohistochemical studies in human cancers have frequently shown that a proportion of invasive carcinomas and carcinomas *in situ* show aberrant levels of E-cadherin and/or catenin expression in comparison to their related normal tissue[17–19]. In general, E-cadherin and catenin staining is strong in well differentiated cancers that maintain their cell adhesiveness and are less invasive, but is reduced in poorly differentiated tumours which have lost their cell–cell adhesion and show strong invasive behaviour. Changes in E-cadherin expression may, therefore, be an important step in the development and progression of a malignant tumour. *In vitro* studies have shown various human cancer cell lines with an epithelioid, differentiated morphology to be generally non-invasive and to express E-cadherin, whereas cell lines with a fibroblast-like morphology are invasive and have often lost E-cadherin expression[20]. Reconstruction of cadherin binding in human carcinoma cell lines by transfection with E-cadherin complementary DNA (cDNA) results in a more differ-

entiated phenotype and loss of invasiveness[20–22]. Furthermore, non-transformed Madin-Darby canine kidney epithelial cells, as well as well differentiated colon carcinoma cell lines, acquire a dedifferentiated and invasive phenotype when intercellular adhesion is inhibited by anti-E-cadherin monoclonal antibodies[23,24]. This clearly demonstrates that E-cadherin can suppress the overt features of advanced tumour progression. However, it remains unsolved whether the loss of E-cadherin-mediated cell adhesion is a prerequisite for tumour progression or is a consequence of dedifferentiation during tumour progression *in vivo*. This question was recently addressed using a transgenic mouse model of pancreatic β-cell tumorigenesis. Perl *et al.*[25] demonstrated that the loss of E-cadherin-mediated cell–cell adhesion is causally involved in the transition from adenoma to invasive carcinoma.

Loss of cadherin-mediated adhesion may also act by promoting tumour cell detachment from the primary site, resulting in dissemination of malignant cells to distant organs. Direct evidence implicating E-cadherin in the development of metastases is based on the association between highly metastasizing carcinomas and low E-cadherin immunoreactivity[26,27]. An *in vitro* study has shown that E-cadherin-negative tumour cells are more likely than E-cadherin-positive cells to be dislodged from the primary tumour by low shear forces, such as these found in venules or the lymphatic system[28]. The involvement of E-cadherin in metastasis was studied using an *in vivo* model of nude mice[29,30]. Injection of E-cadherin-negative breast cancer cells into the circulation gave rise to multiple lung and osteolytic bone metastases in these mice. However, breast cancer cells that were transfected with E-cadherin cDNA showed a significantly impaired capacity to form osteolytic metastases[29]. It is also known that patients with breast cancer whose tumours have reduced E-cadherin expression have a higher frequency of lymph node and distant organ metastases than those whose tumours have preserved E-cadherin expression[27].

E-cadherin as a growth suppressor

It has recently become clear that E-cadherin is involved in contact inhibition of cell growth by inducing cell cycle arrest[1,31]. Sequential activation and inactivation of a family of cyclin-dependent kinases govern the cell cycle. One such cyclin-dependent kinase inhibitor is *p27*, which results in cell cycle arrest; it is now established that E-cadherin has the ability to inhibit cell proliferation by the upregulation of p27[1], although the mechanism by which E-cadherin regulates p27 is still unclear. Inhibiting the activity of mitogenic pathways, perhaps via the epidermal growth factor (EGF) receptor (EGFR), which in turn regulate the level of p27 in cells is suggested. Therefore, E-cadherin, generally described as an invasion suppressor[21], is also a major growth/proliferation suppressor. This attractive model for E-cadherin might explain earlier findings by Hermiston *et al.*[32,33]. They showed that inactivation of E-cadherin in intestinal crypt cells leads to formation of adenomas, and that forced expression of E-cadherin suppresses proliferation.

Role of β-catenin in signal transduction

An important function of β-catenin, namely its role in cell signalling, has been elucidated in the past few years[34–36]. β-Catenin is the vertebrate homologue of the *Drosophila* segment polarity gene armadillo, an important element in the Wingless/Wnt (Wg/Wnt) signalling pathway. Wingless is a cell–cell signal in *Drosophila* that triggers many key developmental processes, Wnt being the vertebrate homologue[37]. In the absence of a mitotic signal from outside the cell β-catenin is sequestered in a complex with the adenomatous polyposis coli (APC) gene product, a serine threonine glycogen synthetase kinase (GSK-3β) and an adapter protein axin (or a homologue conductin), enabling phosphorylation and degradation of free β-catenin by the ubiquitin–proteasome system[38]. The function of and interactions between the proteins in the complex was something of a mystery until recently. Axin, a recently recognized component of the complex, acts as a scaffold protein in the multiprotein structure[39]. Formation of an axin regulatory complex is critical for GSK-3β activity and β-catenin phosphorylation and degradation, since GSK-3β does not bind directly to β-catenin but requires the presence of axin, which binds to both proteins[40]. This complex formation leads to the maintenance of low levels of free cytoplasmic β-catenin. Residual catenins hold cells together by binding to cadherins, both at the adherens junctions and the actin cytoskeleton.

When a mitotic signal is delivered by the Wnt pathway, by association of the Wg/Wnt family of secreted glycoproteins and their membrane receptor frizzled, it leads to activation of the dishevelled (Dsh) protein, which is recruited to the cell membrane. The activated Dsh down-regulates the protein complex, so that it can no longer phosphorylate β-catenin, which then is not degraded[41]. How exactly Wnt signalling leads to the stabilization of β-catenin remains unclear, although it is suggested that that a critical step might be the dissociation of GSK-3β from axin with the help of Dsh[42]. With GSK-3β no longer bound to axin, it cannot phosphorylate β-catenin, leading to an increase in β-catenin levels. Another proposed model is that inhibition of GSK-3β activity upon Wnt signalling by Dsh leads to the dephosphorylation of axin, resulting in a

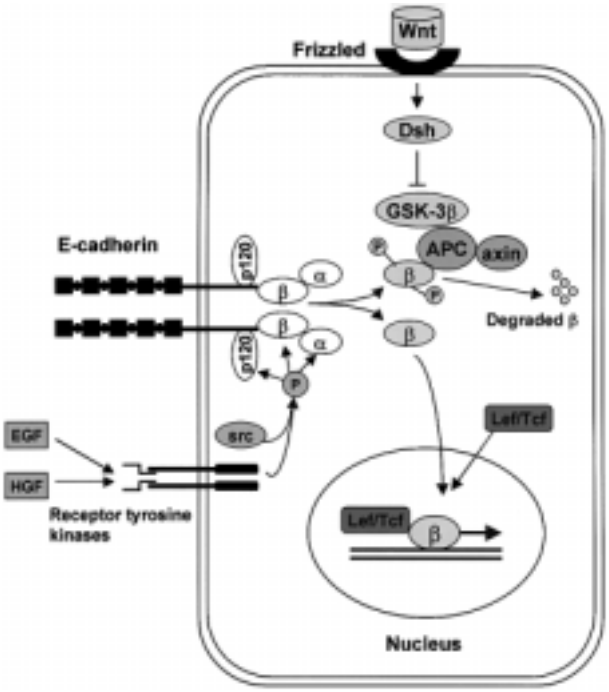

Fig. 2 Schematic representation of the catenins and the interaction with other transmembrane, cytosolic and nuclear proteins. The unbound cytoplasmic pool of β-catenin is sequestered in a protein complex and phosphorylated by glycogen synthetase kinase (GSK-3β, enabling its degradation. Abnormalities in this degradation pathway (activation of the Wnt pathway, mutated adenomatous polyposis coli (APC) gene product or mutated β-catenin) can lead to an increase in the level of free β-catenin, which then translocates to the nucleus, binds transcription factors (Lef/Tcf) and stimulates transcription of target genes. The catenins also interact with receptor tyrosine kinases, such as the epidermal growth factor (EGF) and hepatocyte growth factor (HGF) receptors, which alter their phosphorylation status and, as a consequence, modulate E-cadherin–catenin cell–cell adhesion. See text for details. Dsh, dishevelled

reduced efficiency of binding to β-catenin. The release of β-catenin from the phosphorylation and degradation complex promotes β-catenin stabilization and signalling[43,44]. The resulting increase in free cytosolic β-catenin then enters the nucleus. This results in an increase of free cystolic β-catenin which translocates to the nucleus and directly binds the transcription factors Lef and Tcf, leading to the activation of gene expression[45–47]. Recently, the target genes of these transcription factors have been identified. They are thought to be involved in inhibiting apoptosis and promoting cellular proliferation and migration, and include the c-*myc* oncogene and one of the cell cycle regulators cyclin D1[48–51]. γ-Catenin can also enter the nucleus and interact with these transcription factors, but γ-catenin has been shown to have transactivation capacities different to those of β-catenin, thus leading to distinct signalling properties[52].

Although β-catenin performs distinct functions in E-cadherin-mediated cell–cell adhesion and in Wnt signalling[53], there appears to be some cross-talk between the adhesive and signalling pathways. The expression of excess E-cadherin interferes with Wnt signalling by competing for β-catenin binding[54]. However, loss of cadherin binding does not lead to accumulation of β-catenin free to bind Tcf/Lef-1 and to modulate transcription[55]. Interestingly, Lef^{-1}-β-catenin is also able to bind the E-cadherin promoter, possibly leading to downregulation of E-cadherin[56].

Activation of the Wg/Wnt pathway in human cancers

It is now believed that transformation of adult mammalian cells into malignant tumours reflects an exaggeration of the Wg/Wnt pathway, at least in some tumours. The *APC* gene is mutated in many cancers. In fact, it is supposed to be the first genetic alteration in the multistep process in inherited (familial adenomatous polyposis coli) and sporadic colorectal cancers[57]. Recently, it was found that APC regulates levels of free β-catenin in the cell, as discussed above. Most of the mutations in *APC* result in truncated APC protein which can still complex with, but not degrade, β-catenin[58]. The result of *APC* mutation is, therefore, an increase in cellular free β-catenin, which may trigger a cascade of events resulting in the initiation of adenomas[59]. *APC* mutations in colorectal cancers have been shown to result in an increase in β-catenin/Tcf-4 signalling, leading to overexpression of the c-*myc* proto-oncogene and promotion of neoplastic growth[48,60]. However, somatic mutations or small deletions (but no germline mutations) in the β-catenin gene, targeting the serine and threonine residues necessary for phosphorylation by GSK-3β and breakdown by the APC/GSK complex, are also associated with colon carcinogenesis[60,61]. These β-catenin mutations have a dominant effect, suppressing the APC-dependent binding and degradation of β-catenin, leading to accumulation of cytoplasmic free β-catenin, again with subsequent translocation of β-catenin to the nucleus.

In the past 2 years many studies have revealed that activation of the Wg/Wnt pathway by mutated β-catenin is a rather frequent event in malignancies, including hepatocellular and thyroid carcinomas, desmoid tumours and pilomatricomas[62–65], but uncommon in melanomas, head and neck cancers, and oesophageal and prostatic carcinomas[66–69]. This implicates β-catenin as an oncogene in cancer development, giving a possible explanation for the uncontrolled proliferation observed in cancers. Perhaps this is why widespread nuclear and cytoplasmic staining of β-catenin in colorectal cancers is an independent prognostic

factor for short survival[70]. Similarly, mutations in GSK-3β, axin or other (yet unknown) elements involved in the Wnt/Wg pathway may contribute to β-catenin signalling. Future studies need to focus on these other components of the complex to elucidate the Wg/Wnt pathway in human cancers.

Mechanisms of inactivation of the E-cadherin–catenin complex in cancer

Human cancers possess both irreversible and reversible mechanisms for inactivation of the E-cadherin–catenin complex which lead to invasion and metastases. Loss of the E-cadherin locus on the long arm of chromosome 16 (16q22) occurs at rather high frequency in hepatocellular (50 per cent), lobular breast (50 per cent) and oesophageal (66 per cent) carcinomas[71–73]. There have been several reports on E-cadherin gene mutations in human cancers[74]. In poorly differentiated tumours, such as lobular breast cancer and diffuse-type gastric cancer, E-cadherin mutations play an important role in tumour development[75,76]. However, mutations are rare in oesophageal, thyroid and colorectal carcinomas[73,77,78]. Interestingly, E-cadherin mutations with loss of the remaining wild-type allele are also detectable in the preinvasive *in situ* component of lobular breast cancer and in intramucosal lesions of gastric signet ring cell carcinomas[79,80]. This, together with the findings of Perl *et al.*[25], indicates that genetic alterations of the E-cadherin gene are involved in the early developmental stages of some histological types of human cancer, and that both E-cadherin alleles are inactivated. The importance of loss of function of E-cadherin in the onset of cancer is now well established. Several studies have reported germline mutations in the E-cadherin gene in families with an inherited diffuse type of gastric cancer[81,82]. However, the clinical implications of these findings are not yet clear. Only a minority of gastric cancers can be accounted for by E-cadherin mutations. Recently, it has been recognized that early onset of lobular breast carcinoma may also be associated with E-cadherin germline mutation[82,83].

So far, mutations in the α-catenin gene have been described only for cell lines, not in tumours *in vivo*[84,85]. Human cultured cancer cell lines with a genetically altered α-catenin only regain their cell–cell adhesiveness when transfected with wild-type α-catenin cDNA[86]. Therefore α-catenin meets the criteria of an invasion suppressor gene.

Genetic alterations in β-catenin abolishing cell–cell adhesiveness have been observed in two gastric cancer cell lines, HSC 39 and 40A; both derive from the same signet ring cell carcinoma of the stomach and show a diffuse growth pattern[87,88]. This mutation results in a truncated β-catenin that lacks the region for interaction with α-catenin. Transfection of these cell lines with wild-type β-catenin restores cellular adhesiveness[88]. Recently, a mutation in γ-catenin has been described in a gastric cancer cell line, but no mutations have been reported in sporadic gastric cancers[55].

Downregulation of E-cadherin expression may be induced by a low activity of the E-cadherin promoter owing to chromatin rearrangement in the regulatory domain or to DNA methylation[89,90]. *In vivo* experiments show that methylation in the E-cadherin promoter region correlates significantly with reduced E-cadherin expression in hepatocellular carcinomas and methylation may also be detected frequently in precancerous conditions[91]. Furthermore, an *in vitro* experiment has shown that downregulation of E-cadherin by stimulating c-erbB2 transcription further reduces E-cadherin promoter activity, suggesting a role for c-erbB2 overexpression in tumour progression and metastases[92]. However, an immunohistochemical study has produced a strong argument against a role for c-erbB2 as a transcriptional regulator of E-cadherin expression in breast carcinomas *in vivo*[93]. In addition, downregulation of β-catenin in cholangiocarcinomas is associated with c-erbB2 downregulation[94]. EGFR and the tumour suppressor p53 may also play a role in the regulation of E-cadherin and α-catenin expression, and perturbation of the E-cadherin–catenin complex[95,96]. It is also known that *Helicobacter pylori* infection is associated with downregulation of E-cadherin in gastric mucosa and so might play a role in the onset of neoplastic growth[97].

In some tumours, including pancreatic, thyroid, gastric, bronchopulmonary, oesophageal, colorectal and bladder cancers, the staining pattern of the E-cadherin–catenin complex does not always indicate an absence or reduction in expression but shows a redistribution from the cell membrane to the cytoplasm[19,98–103]. The mechanism responsible for this redistribution in tumour cells remains elusive. Immunoprecipitation experiments have been performed to address this point, with modifications in the interactions between E-cadherin and the catenins being observed[98,104]. In colorectal tumours with heterogeneous cytoplasmic immunoreactivity for E-cadherin as well as the catenins, one of the catenins was not present in the complex, or the cytoskeletal bound fraction was reduced in spite of an overall increase in expression of the proteins (M. El-Bahrawy, personal communication)[99,105]. These studies have shown that the expression of the proteins does not necessarily imply that they are functioning; binding of the E-cadherin–catenin complex to the cytoskeleton is essential for its role in cell adhesion.

Failure of E-cadherin and catenins to localize to the membrane and/or bind to the cytoskeleton in spite of their abundant presence may be due to alterations in their phosphorylation status[100,101]. A number of receptor and

non-receptor tyrosine kinases and phosphatases, including the EGFR, the c-*erbB2* oncogene and the hepatocyte growth factor receptor, c-met, interact with the catenins. This interaction alters the phosphorylation status of the catenins and, as a consequence, cadherin-mediated adhesion[106–110]. For example, EGFR has tyrosine kinase activity that is activated through autophosphorylation upon its binding to EGF. By this mechanism EGF induces immediate phosphorylation of β- and γ-catenin which is inhibited by herbimycine, a tyrosine kinase inhibitor[111]. Overexpression of EGFR, c-erbB2 and c-met has been described in several cancers. This was associated with cellular redistribution of E-cadherin from the membrane to the cytoplasm along with suppression of its function[111,112]. In addition, there is evidence to suggest that the association between E-cadherin and α-catenin can be prevented by tyrosine phosphorylated β-catenin[113].

Further evidence of the possible role of tyrosine phosphorylation as a mechanism by which E-cadherin–catenin function is modulated comes from findings in cells transfected with the v-*src* oncogene. Increased tyrosine phosphorylation of β-catenin and E-cadherin is observed and this post-translational modification results in functional changes, such as decreased cell–cell adhesion, increased migration and increased invasiveness, without affecting the overall expression of either of the catenins or the cadherins[114,115]. The inhibition of tyrosine phosphorylation restores cadherin function to normal[114]. Other studies show that upregulation of tyrosine phosphorylation of β-catenin and p120 occurs frequently in surgical specimens of colorectal and lung cancer, and that phosphorylation of β-catenin correlates well with poor survival of patients after surgery[116,117]. These results suggest that tyrosine phosphorylation of the catenins might be a significant mechanism that modulates their function and, in turn, that of E-cadherin–catenin; this may have important prognostic value.

E-cadherin–catenin expression in cancer and its possible clinical relevance

Immunohistochemical studies of many different types of human carcinomas (including skin, head and neck, lung, breast, thyroid, oesophageal, gastric, pancreatic, hepatocellular, colon, renal, bladder, prostate, endometrial and ovarian carcinomas) have shown that a proportion of these neoplasms have reduced levels of E-cadherin expression in comparison to their related normal tissues. Indeed, E-cadherin loss is most pronounced in those types of carcinoma that have strikingly infiltrative growth patterns associated with little or no intercellular cohesion, such as invasive lobular breast cancer and diffuse-type gastric adenocarcinoma. Therefore, it is not surprising that abnormal expression of the E-cadherin–catenin complex correlates with pathological characteristics of the tumour, such as grade of differentiation, invasiveness, venous permeation, peritoneal seeding, lymph node involvement, liver and bone metastases and tumour stage[16,118–124]. Interestingly, aberrant expression of E-cadherin, α-, β- and γ-catenin, and p120 correlates with clinical variables, such as disease relapse, disease-free survival and overall survival[121,125–131]. Moreover, aberrant expression of E-cadherin and/or the catenins has been shown to be an independent prognostic marker for shorter survival, although its predictive value is usually less strong than that of the standard variables such as tumour grade, tumour stage and lymph node metastases[128,132–136]. Of particular interest is the finding that E-cadherin is an independent predictor of occult lymph node metastasis and micrometastases in nodes classified as N_0 by routine histopathological methods[137]. This is in accordance with studies that show an additive value for E-cadherin–catenin expression in patients with no signs of lymph node or distant metastases (N_0 and M_0)[131,138]. Immunohistochemical detection of E-cadherin and the catenins might be useful not only in predicting disease-free or overall survival but also in identifying patients with clinically negative lymph nodes who are at risk of occult metastases and who may benefit from more extensive lymph node dissection.

A number of studies have failed to show a relationship between E-cadherin–catenin expression and clinicopathological variables[139,140]. Several explanations for this discrepancy can be suggested. The histological type and number of cancers analysed, selection of the tumours (stage, tumour grade), demographics of the study population[141], differences in the surgical approach (extent of lymph node dissection)[118,142], and differences in staining evaluation may individually or in combination be held responsible.

Some studies report that the combination of E-cadherin and one of the catenins is of better prognostic value than evaluation of the individual components[27,143]. It is important to note that alterations in any component may lead to disrupted function of the complex. Since catenins play a critical role in the regulation of cadherin-mediated adhesion, this indicates that E-cadherin immunoreactivity does not always imply the presence of a functionally normal cadherin–catenin complex. Thus, to predict tumour invasion and metastasis in carcinomas, it is useful to investigate not just the expression of E-cadherin but also the expression of the catenins. In addition, these results re-emphasize the importance of understanding the regulatory pathways of cell adhesion in order to interpret correctly the immunohistochemical data on adhesion molecule expression in tumours. Interestingly, the lack of correlation between

cadherin and catenin immunoreactivity is also consistent with the promiscuous and yet selective association of catenins not only with E-cadherin, but also with other transmembrane (e.g. EGFR), cytosolic (e.g. APC) and nuclear (Tcf) proteins. Large clinicopathological studies evaluating all members of the adhesion complex within a well defined population, with complete follow-up, are needed to validate the use of E-cadherin and catenins as predictors of tumour cell behaviour.

Alterations in E-cadherin and catenin expression have also been found in preinvasive lesions of the colon (adenoma), oesophagus (Barrett's dysplasia), stomach (gastric dysplasia) and breast (ductal carcinoma *in situ*)[18,19,144,145]. In addition, raised levels of a soluble form of E-cadherin (sE-cadherin; 80 kDa) can be detected in the serum of patients with cancer, possibly induced by tumour-associated proteolytic degradation. In those with bladder cancer a correlation exists between the level of sE-cadherin and grade, stage of the tumour and with tumour recurrence on cystoscopy[146,147]. Furthermore, sE-cadherin may serve as a tumour marker with a rather high sensitivity compared with CA19–9 and carcinoembryonic antigen in patients with gastric cancer[148]. In ovarian carcinoma E-cadherin levels show no correlation with the response to chemotherapy or 5-year survival. Therefore, it may be concluded that determination of preoperative levels of sE-cadherin does not offer useful clinical information for the management of patients with ovarian cancer[149]. Undoubtedly, more evidence from larger studies is needed to address the possible usefulness of sE-cadherin as a disease marker for cancer.

A combination of decreased E-cadherin expression with altered expression of other proteins involved in cancer invasion and metastasis has been described. For example, a combination of decreased E-cadherin expression with upregulation of urokinase-type plasminogen activator (a protease involved in cancer invasion and metastasis) is an independent predictor of prognosis in patients with gastric cancer[150]. Simultaneous reduced expression of E-cadherin and CD44v6 in breast cancer, and low E-cadherin expression in combination with high CD44s in renal cancer, correlates with poorer survival[129,151]. Combination of low E-cadherin immunoreactivity and high type IV collagenase expression is an independent predictor of disease recurrence and overall survival, but not of stage, nodal metastases and histological type, in pancreatic cancer[152]. Finally, Otto *et al.*[118] found that abnormal expression of both E-cadherin and gp78 (the receptor for a tumour-derived autocrine motility factor) in patients with bladder carcinoma results in poor outcome, independent of tumour stage and grade.

Recent studies suggest that modulation of E-cadherin and catenins may be more complex than previously thought. For example, it has been demonstrated that in 40 per cent of adenocarcinomas E-cadherin levels are raised in their intravascular tumour components in comparison to their extravascular compartments[153]. Moreover, expression of E-cadherin or the catenins has been found to be higher in lymph node metastases than in the primary tumour[122,136,145] and, surprisingly, there is a greater tendency for liver metastasis in tumours in which the integrity of E-cadherin-mediated cell adhesion is intact[154]. This strongly suggests that the levels of cadherin–catenins are not necessarily fixed, but in an appropriate microenvironment may be subject to transient manipulation. One explanation may be that entrance of a carcinoma into an intravascular compartment is associated with an upregulation of E-cadherin expression, and that subsequent exit into extravascular tissues is associated with downregulation[155]. Second, upregulation of E-cadherin expression at implantation sites may be necessary for tumour cells to cluster and grow out as metastases. These concepts are supported by *in vitro* data, and the fact that the staining pattern of E-cadherin in human tumours is often heterogeneous and unstable[156,157].

E-cadherin–catenin: a target for anticancer therapy?

Since the function and expression of the E-cadherin–catenin complex is often reduced in cancer cells, it is suggested that restoration of the E-cadherin–catenin will lead to differentiation and anti-invasive properties. Several drugs have been described to alter the expression of E-cadherin, some of which are already used in the treatment of cancer. At least *in vitro*, insulin-like growth factor 1, tamoxifen, taxol, retinoic acid and progestagens have been shown to upregulate the functions of the E-cadherin–catenin complex, including inhibition of invasion[158–162]. The mechanisms by which these drugs induce these changes include upregulation of E-cadherin, α- and β-catenin messenger RNA expression, dephosphorylation of β-catenin, increased stability of the β-catenin protein, and localization of β-catenin at the cell–cell junctions[160,162–164]. A few more drugs may be added to the list of compounds that restore E-cadherin–catenin-dependent cell–cell adhesion; aspirin is probably the most intriguing[165]. Epidemiological, animal model and clinical studies all suggest that non-steroidal anti-inflammatory drugs are potent preventive agents against colon cancer. Mahmoud *et al.*[165] showed that aspirin decreased the rate of tumour formation in MIN mice; these mice have a germline mutation in *APC* which leads to an increase in cytoplasmic β-catenin with subsequent cell signalling. Aspirin produces a decrease in intracellular β-catenin levels, suggesting that modulation of this protein is associated with tumour prevention.

Table 1 E-cadherin–catenin complex: clinical findings and implications

Findings	References
Expression of E-cadherin–catenin correlates with histopathological findings: tumour grade, invasion and wall infiltration, venous permeation, lymph node, liver and bone metastases, tumour stage	26,118–124
Expression of E-cadherin–catenin can predict survival after surgery: overall 5-year survival, 5-year disease-free survival, disease relapse	121,125–131
Expression of E-cadherin can predict occult lymph node metastases/micrometastases	131,137,138
Malignant degeneration: normal epithelium→dysplasia→carcinoma sequence	18,19,144,145
Soluble E-cadherin in sera of patients with cancer could serve as a tumour marker: follow-up of patients, detecting recurrence, response to (adjuvant) therapy?	146–148
Identifying families with hereditary diffuse type of gastric cancer (and lobular breast cancer?)	81,82
Anticancer therapy and cancer prevention: restoring abnormal expression of E-cadherin–catenin by drugs	158–162,165

Conclusions

Inactivation of the E-cadherin–catenin cell–cell adhesion complex is mediated by genetic and epigenetic events that occur in both the early and late stages of carcinogenesis. These molecules may be useful in assessment of the malignant potential of preinvasive lesions and the development of prognostic markers for cancer. *Table 1* summarizes the relevant clinical findings and their possible implications. Apart from epithelial cell–cell adhesion, E-cadherin–catenin is involved to a much wider extent in cancer cell biology. Surprising findings regarding the interaction between APC and β-catenin, and its role in cell signalling, have clearly shown that this complex has a key role in malignant cell transformation. Moreover, elucidation of the mechanisms underlying the changes in E-cadherin and catenin function may lead to the development of novel therapeutic approaches based on biochemical and genetic manipulation.

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