

Gut

Leading article

The importance of the E-cadherin-catenin complex in the maintenance of intestinal epithelial homeostasis: more than intercellular glue?

Introduction

The intestinal epithelium is characterised by rapid cell turnover, with pluripotential stem cells in the crypts of Lieberkühn providing a continuous supply of cells which are directed into a variety of maturation pathways. Development and maintenance of normal intestinal epithelial morphology requires regulation of daughter cells' proliferative status, lineage allocation, migration, differentiation, and apoptosis.¹ It has become increasingly evident that co-ordination of these processes and the consequent generation of the characteristic intestinal epithelial architecture are highly dependent on intercellular and cell-matrix adhesive interactions.² These adhesive events are not random, but through selective interactions, organise cells into diverse and highly distinctive patterns. Connections of cell junctions such as desmosomes, hemidesmosomes, and adherens junctions to the actin cytoskeleton, thus allow maintenance of cell polarity and tissue architecture.³ Variation in the functional state of these adhesive mechanisms is critical to the dynamic processes necessary for tissue morphogenesis in the embryo,⁴ where many morphogenetic events are correlated with a unique spatiotemporal pattern of cadherin expression.⁵ Such variation is also critical to the maintenance of this highly complex architecture in various physiological and pathological states, such as migration of cells up the crypt-villus axis⁶ and repair of mucosal injuries. In view of these facts, it is not surprising that many cell adhesion molecules have been implicated in cell signalling pathways.^{7–8}

E-cadherin: a cell-cell adhesion receptor

E-cadherin is a member of the large cadherin superfamily and is the predominant intercellular adhesion molecule expressed by intestinal epithelial cells.³ It is a calcium dependent transmembrane protein which forms a key component of adherens junctions. E-cadherin molecules form dimers at the cell surface, which interdigitate with other E-cadherin molecules on adjacent epithelial cells resulting in the formation of cell adhesion "zippers" (fig 1).^{9–10} E-cadherin has classically been thought to be exclusively involved in homotypic cell-cell interactions; however, evidence has emerged suggesting a heterophilic interaction with $\alpha_E\beta_7$ integrin on the surface of lymphocytes. $\alpha_E\beta_7$ is expressed predominantly on intra-epithelial lymphocytes, but on only a minority of circulating lymphocytes, and this interaction is therefore suggested to be

important in mediating the retention of lymphocytes within the epithelium in a tissue specific fashion.¹¹

The critical importance of E-cadherin to normal development and tissue function is demonstrated by the lethality of E-cadherin gene knockouts in mice, at a very early stage in embryogenesis.^{12–13} Reduced or absent expression of E-cadherin has been described in a variety of epithelial malignancies due to mutation, hypermethylation of the E-cadherin promoter,¹⁴ abnormal transcription, or post-transcriptional modification by tyrosine phosphorylation.^{15–16} These abnormalities are associated with aggressive histopathological characteristics such as increased tumour invasiveness and metastasis,^{17–18} and in some cases with poor survival,^{19–20} leading to the suggestion of a role for E-cadherin as a tumour suppressor.^{21–22}

The catenins

The functions of E-cadherin are mediated through its linkage to the actin cytoskeleton via a number of cytoplasmic plaque proteins known as the catenins (fig 1).²³ The catenins seem to be critical both to the function of cadherin mediated adhesion²⁴ and to the transduction of signals initiated at the cell surface by the adhesion receptors.²⁵ β -catenin forms a complex with E-cadherin, while α -catenin links this complex to the actin cytoskeleton.²⁶ β -catenin shows a high degree of homology to *armadillo*, a segment polarity gene in *Drosophila*, which is an essential component of the WNT signalling pathway that controls developmental patterning in both *Drosophila*²⁷ and *Xenopus* embryos.²⁸ β -catenin undergoes tyrosine phosphorylation in response to growth factor stimulation resulting in reduced adhesion,²⁹ and is therefore thought to act as a regulatory component of the cadherin-catenin complex, and to link signal transduction to intercellular adhesion. Disruption of the β -catenin gene prevents normal development of embryonic ectoderm, and causes early lethality.³⁰ Finally, γ -catenin bears homology to β -catenin and is identical to desmosomal plakoglobin. β - and γ -catenins appear to form mutually exclusive complexes with E-cadherin,³¹ although both form similar interactions with a number of proteins implicated in signal transduction, control of proliferation and pathogenesis of neoplasia, including the epidermal growth factor receptor (EGFr),³² the oncogene *c-erbB2*,³³ and the adenomatous polyposis coli (APC) protein product.^{34–35}

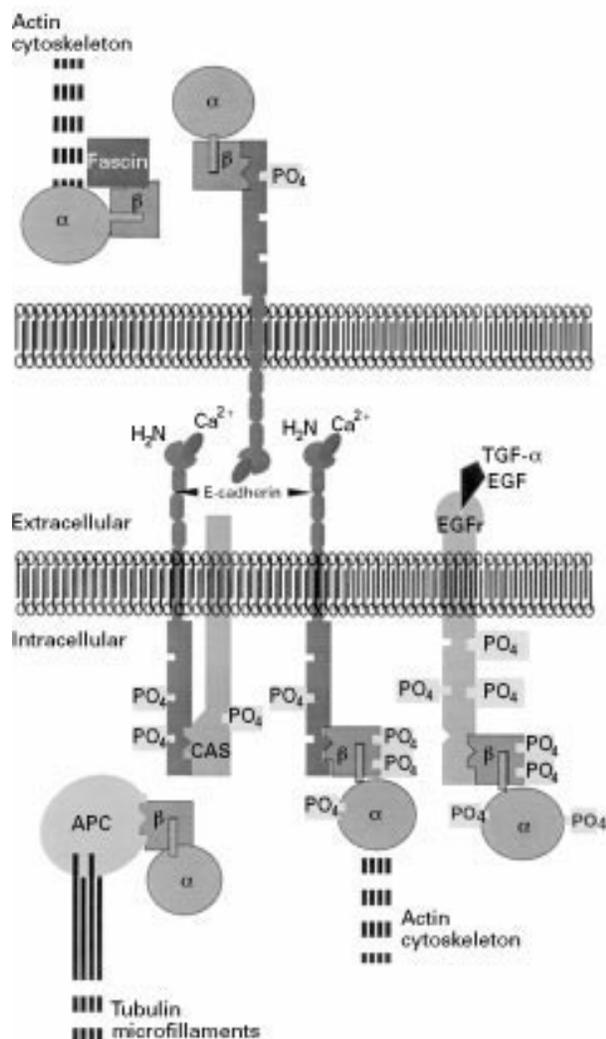


Figure 1: Multimolecular complexes formed by catenins. E-cadherin is a transmembrane protein which binds to β - or γ -catenin in a mutually exclusive interaction. α -catenin links this complex to the actin cytoskeleton. p120^{cas} binds to the cytoplasmic domain of E-cadherin without any link to the cytoskeleton. Other complexes formed by β -catenin include an interaction with the actin bundling protein fascin (top left), a complex with the adenomatous polyposis coli (APC) protein, and in turn to the tubulin microfilament network (bottom left), and another with the epidermal growth factor receptor (EGFr) (bottom right). TGF- α , tumour growth factor α .

The role of the E-cadherin-catenin complex in maintenance of epithelial morphology and homeostasis

In vitro cell culture studies have suggested an important role for the cadherin-catenin complex in many processes including regulation of cell polarity, formation of junctional complexes, cell migration and proliferation. Further understanding of its role in vivo, however, has been hampered by the complexity of cell adhesion systems, and the problem of early lethality of E-cadherin and β -catenin gene knockouts. In a series of very elegant experiments, Hermiston *et al* recently overcame these problems and demonstrated the crucial role of E-cadherin in providing instructive cell-cell interactions which influence cell fate including cell migration, proliferation and apoptosis in the intestinal epithelium.^{36, 37} They transfected 129/Sv embryonic stem cells with a recombinant DNA containing the gene of interest (E-cadherin, or N-cadherin null mutant), placed under the control of a promoter active at selected locations along the intestine's crypt-villus axis and

duodenal-ileal axis. The transfected cells were then introduced into mouse B6 blastocysts resulting in chimaeric mice, which express two populations of monoclonal crypts—normal or transgenic. The authors demonstrated that expression of a dominant negative N-cadherin mutant in the crypt-villus axis resulted in disruption of intercellular and cell-matrix contacts, with an increased cell migration rate up the villus, loss of cell differentiation and polarisation, and precocious apoptosis, confirming the importance of intact E-cadherin mediated adhesion in regulating cell fate and maintenance of normal epithelial homeostasis.

An intact cadherin-catenin complex has been shown to be important in the development of functional tight junctions³⁸ which are necessary for the maintenance of the selective permeability and barrier function of the intestinal epithelium. It is therefore perhaps not surprising that by 6 weeks of life, all chimaeric mice demonstrated increased inflammatory cell infiltration in the transgenic areas of intestinal epithelium, which showed evidence of epithelial barrier disruption. Transmural inflammation with a clinical picture analogous to Crohn's disease was seen by 3 months of age. A hyperproliferative state was noted in the crypts with early development of adenomas and dysplastic change, although progression to carcinoma was not observed in the first 19 months of life.

A role in epithelial cell migration

The importance of cell-matrix adhesive interactions in controlling cell migration has been extensively studied.³⁹ The extending lamellipodium of the migrating cell binds to the substratum through integrin receptors. These adhesive interactions are then used to generate the traction force required for cell movement, followed by release of adhesions at the rear of the cell. Less is known, however, about the role of the E-cadherin-catenin complex in this process. In both families of cell adhesion molecules, control of cell migration is thought to involve intimate interactions between the transmembrane adhesion receptors and the cytoskeletal motility apparatus.

Recent data have demonstrated binding of β -catenin to fascin,⁴⁰ and of α -catenin to α -actinin,⁴¹ both of which are actin bundling proteins.⁴² These proteins are known to be important in the dynamic assembly and organisation of actin bundles and networks necessary for the extension of lamellipodia, the first step in cell migration. This interaction between proteins involved in intercellular adhesion and those controlling motility, supports the notion of co-ordinate regulation of cadherin mediated adhesive interactions and cell motility. Such regulatory co-ordination is particularly important under certain physiological circumstances such as mucosal repair by epithelial restitution.⁴³ Downregulation of E-cadherin expression or function to permit motility of regenerative epithelium over ulcers is one potential mechanism⁴⁴ which may be facilitated by factors influencing binding of E-cadherin to β -catenin. Phosphorylation of β -catenin by type I tyrosine kinase growth factor receptors, such as the EGFr, may be one such mechanism.²⁹ Alternatively, changes in the availability of β -catenin for binding to E-cadherin is another. β -catenin forms complexes in the cytoplasm with APC, the adenomatous polyposis coli protein product, which competes with E-cadherin for binding.⁴⁵ Wild type APC has been shown to downregulate cytoplasmic β -catenin levels,⁴⁶ and to have an important role in microtubule dependent cell migration.⁴⁷ The carboxy-terminal domain of the wild type APC protein, which effects the downregulation of β -catenin, is truncated in tumours containing APC gene mutations.⁴⁷ Hinck *et al* have shown that a dynamic equilibrium exists between

E-cadherin/ β -catenin complexes, free pools of catenins and APC/ β -catenin complexes.⁴⁸ The actin bundling protein fascin also competes for binding to β -catenin. Factors influencing these interactions may, therefore, alter the stoichiometry in favour of the adhesive complex (E-cadherin/ β -catenin), or the motility complexes (APC/ β -catenin or fascin/ β -catenin)

Signal transduction through the cadherin-catenin complex

An important question in this complex system is the relation between the adhesive functions and signal transduction. One early hypothesis postulated that increased levels of β -catenin promote signalling by increasing cell adhesion through increased juxtacrine interactions.⁴⁹ More recent evidence, however, suggests that E-cadherin itself may be involved in the signalling cascade, by controlling availability of β -catenin in the cytoplasm for signalling. Alternatively, E-cadherin could antagonise the signalling pathway by sequestering β -catenin, resulting in a reciprocal relation between cell adhesion and signalling.^{50–51} Sequestration of β -catenin by E-cadherin could therefore promote increased intercellular adhesion and decreased cell migration with reduced proliferation, while shifting of the equilibrium in favour of cytoplasmic APC/ β -catenin complexes may promote increased cell mobility and proliferation.

Finally, while the precise downstream targets for β -catenin signalling remain unclear, recent data suggest a role for β - and γ -catenin in the regulation of the nuclear transcription factors LEF-1⁵² and Xtcf-3.⁵³ Transfection of LEF-1 or Xtcf-3 together with β - or γ -catenin into previously negative cells is associated with nuclear translocation of the catenins with transcriptional activation. β - and γ -catenins may thus influence the DNA binding or regulatory properties of these transcription factors, thus supporting a role for these proteins in signal induced DNA synthesis and gene expression. These findings have taken on particular significance with respect to understanding the tumour suppressor role of APC in the pathogenesis of colonic neoplasia. APC has been shown to regulate cytoplasmic β -catenin levels through changes in its phosphorylation state, mediated by the APC associated serine threonine kinase GSK-3 β . APC mutations result in accumulation of uncomplexed cytoplasmic β -catenin which is then translocated into the nucleus, leading to uncontrolled transcription of hTcf-4 target genes.⁵⁴ In other words, wild type APC mediates its tumour suppressor function by inhibition of β -catenin mediated activation of the colon specific transcription factor hTcf-4. To support this hypothesis, colonic tumours lacking APC mutations show constitutive activation of these transcriptional factors through an alternative pathway. Such tumours have been shown to harbour β -catenin mutations involving loss of serine phosphorylation sites, which are implicated in the downregulation of β -catenin through the action of the serine threonine kinase GSK-3 β , thus rendering β -catenin insensitive to APC mediated downregulation.

Conclusion

Evidence is accumulating of the central role of E-cadherin mediated adhesion in the development and maintenance of epithelial morphology and maintenance of its homeostasis, through its involvement in signal transduction cascades that regulate epithelial cell fate, including lineage allocation, cell differentiation, migration, proliferation, and apoptosis. The involvement of APC with β -catenin, together with the tumour suppressor function of E-cadherin, support a role for these proteins in tumorigenesis. Further understanding of how these signal cascades are

controlled provides a tantalising area for further research with wide reaching implications in terms of understanding this fundamental area of epithelial cell biology.

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