Review

Modulation of Epithelial Cell Adhesion in Gastrointestinal Homeostasis

Jason Alexander Efstathiou* and
Massimo Pignatelli†

From the Cancer and Immunogenetics Laboratory,* Imperial Cancer Research Fund, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DS, and the Division of Investigative Science,† Imperial College of Science, Technology and Medicine, Hammersmith Campus, Du Cane Road, London W12 0NN United Kingdom

Introduction

From the simple nematode *Caenorhabditis elegans* to the more complex mammal *Homo sapiens*, the gut occupies a well differentiated and dynamic center of activity in the metazoan body. Normal intestinal epithelial cell kinetics is characterized by rapid cell turnover whereby pluripotent stem cells from the Lieberkühn's crypts provide a constant supply of daughter cells that pursue maturation pathways of migration-associated differentiation, polarization, proliferation, programmed cell death, and luminal shedding along the crypt-villus axis (Figure 1).^{1,2} These processes are fundamental for the maintenance of normal intestinal epithelial architecture and function and are dependent on molecules mediating cell-cell and cell-matrix interactions.³

A few main supergene families of transmembrane cellular adhesion receptors have been identified: the cadherins, which mediate interactions between neighboring cells; the integrins, which regulate interactions with the extracellular matrix; the selectins, which are found on leukocytes and endothelial cells; and members of the immunoglobulin and proteoglycan superfamilies.^{3,4} In this article we focus our attention primarily on epithelial cadherin (E-cadherin) and its associated cytoskeletal partners, the catenins, and the regulatory role they play in the behavior of normal and restituting gastrointestinal epithelial cells. We also give some consideration to the importance of integrins and suggest the possibility for cross-talk between the cadherin- and integrin-mediated adhesion systems.

A number of clinicopathological studies have revealed qualitative and quantitative changes in cadherin-catenin⁵ and integrin⁶ expression and function in a variety of epithelial malignancies. Hirohashi, in this issue,⁷ elucidates

the importance of E-cadherin-mediated cell adhesion dysfunction in human cancers. We, on the other hand, examine modulation of the E-cadherin-catenin adhesion complex during other pathophysiological processes of the gastrointestinal tract including embryogenesis, differentiation, cell migration, and the repair of mucosal damage.

Cell-Cell and Cell-Matrix Adhesion

E-cadherin is localized mainly to the zonula adherens junctions and serves as the prime mediator of epithelial cell-to-cell adhesion through calcium-dependent, homotypic interactions.⁸ E-cadherin's cytoplasmic tail associates with the catenins (α -, β -, γ - and p120), a family of closely related but distinct membrane undercoat proteins, which link to the actin microfilament network of the cellular cytoskeleton.⁹ Such binding is essential for the adhesive function of E-cadherin.⁵

The integrins are also a family of transmembrane glycoproteins, but exist as non-covalently associated α/β chain heterodimers and are associated with the actin cytoskeleton through linker proteins such as talin, vinculin, and α -actinin.^{6,10} Most of the integrins function as cell surface receptors for extracellular matrix components including collagen, laminin, and fibronectin. Whereas integrins are expressed by every cell derived from the three primary germ layers, the gastrointestinal epithelium expresses multiple integrin receptors with overlapping specificity.^{6,10}

Interestingly, there is evidence suggesting a heterophilic interaction between E-cadherin and $\alpha_E\beta_7$ -integrin on the surface of lymphocytes.¹¹ Further evidence for cross-talk between integrins and cadherins comes from cultured keratinocytes in which inhibition of cadherin-mediated adhesion results in decreased integrin expression by proliferating cells and *de novo* expression by differentiating cells.¹² In fact, integrin-mediated cell ad-

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Address reprint requests to Dr. Massimo Pignatelli, Division of Investigative Science, Imperial College of Science, Technology and Medicine, Hammersmith Campus, Du Cane Road, London W12 0NN UK. E-mail: m.pignatelli@rmps.ac.uk.

Figure 1. Schematic representation of an intestinal crypt.

hesion plays an important role in epithelial cell renewal by promoting terminal differentiation of cultured keratinocytes.¹³ Furthermore, both E-cadherin and $\alpha_2\beta_1$ -integrin, the main collagen receptor expressed by epithelial cells, are necessary to induce the morphological differentiation of colorectal tumor cells *in vitro*.¹⁴ In addition, overexpression of *c-erbB2* in human mammary epithelial cells inhibits the transcription of the E-cadherin as well as the $\alpha_2\beta_1$ -integrin gene.¹⁵ Numerous studies involving the addition of neutralizing monoclonal antibodies, gene transfer, and knockout mouse models have confirmed that structural and functional integrity of the components of the E-cadherin-catenin and integrin complexes are necessary during stable epithelial cell adhesion.^{5,6}

Catenin-Mediated Signaling

The catenins and their complexes act not only as intercellular glue but also as integral components of intracellular signal transduction pathways, linking the membrane to downstream cytoplasmic and nuclear events. They have been found to associate promiscuously and yet selectively with cell surface, cytosolic, and nuclear partners¹⁶ which have growth regulatory and signaling functions, such as the epidermal growth factor (EGF) receptor,¹⁷ the oncogene *c-erbB2*,¹⁸ the product of the adenomatous polyposis coli (*APC*) tumor suppressor gene¹⁹ and the Tcf-Lef transcription factors.²⁰ Interestingly, *APC* has been found to compete directly with E-cadherin for binding to β -catenin²¹ and to form distinct complexes containing combinations of the catenins which are independent from the cadherin complexes and through them bind to the cytoskeleton. Further complicating the picture is the recent report that Axin forms a tetrameric complex with *APC*, glycogen synthase kinase (GSK)-3 β , and β -catenin resulting in the phosphorylation-dependent regulation of β -catenin stabilization and degradation.^{22,23} Recently, it has been reported that the

forced expression of a NH₂-terminal β -catenin truncation mutant in a mouse model leads to increased cell division in the proliferative compartment of the intestine and crypt apoptosis.²⁴ This was associated with a marked augmentation of E-cadherin at the adherens junctions and basolateral surfaces of the intestinal epithelial cells as well as a reduction of cell migration along the crypt-villus axis. There is also evidence that *APC* may alter transformation properties, decrease growth rate, or induce apoptosis in colon carcinoma cells.^{25,26} It appears that the maintenance of normal intestinal epithelial cell kinetics may critically depend on coordination among the various membrane, cytosolic, and nuclear catenin pools.

Adhesion Molecules during Development and Differentiation

The expression of cell adhesion molecules is developmentally regulated. A number of model systems have allowed *in vivo* investigation of the role played by cadherins, catenins, and integrins in embryonic tissue development and epithelial differentiation. Mouse knockout experiments, by gene-targeting embryonic stem cells, have confirmed the requirements of cadherins²⁷ and β_1 -integrins²⁸ in stable cell-cell and cell-matrix adhesion. E-cadherin knockouts, for example, revealed that E-cadherin-mediated cell adhesion is essential for the compaction of mesenchymal cells and their transition into a polarized epithelium. E-cadherin null mutant embryos were further associated with lack of trophoblast formation or blastocyst cavity formation and with early embryonic lethality.^{27,29} Similarly, β -catenin knockouts resulted in the absence of mesoderm formation and disturbed ectoderm development and were embryonic lethals.³⁰

In *Drosophila* and *Xenopus* embryos, β -catenin and its *Drosophila* homolog, *Armadillo* (ARM), are involved in the Wnt/Wingless signaling pathway critical for developmental patterning.³¹ Mutation of ARM results in defective segment polarity.³² Recent evidence also suggests a role for the cadherin-catenin system in the normal development of the central nervous system.³³ ARM function was found to be required early in development for determination of neuroblast fate and later in development for the construction of the axonal scaffold.³³ Ectopic overexpression of β -catenin³⁴ or γ -catenin³⁵ by the injection of synthetic mRNA in the future ventral side of an early *Xenopus* embryo mimics the Nieuwkoop center and induces a secondary dorsoanterior axis. The expression of full-length *APC* results in similar duplication of the body axis in *Xenopus* embryos, which has led to the suggestion that *APC* acts as part of the Wnt/ β -catenin signaling pathway.³⁶ Conversely, injection of wild-type Axin blocks axis formation and prevents axis duplication, which suggests that it acts as a negative regulator of the Wnt pathway.³⁷ Depletion of β -catenin by injection of antisense oligonucleotides³⁸ or anti- β -catenin antibodies³⁹ also causes defective dorsal development, while expression of a dominant negative cadherin mutant in *Xenopus* embryos resulted in perturbations of cell adhesion and tissue morphogenesis.⁴⁰

Further evidence for the inextricable link between cell adhesion molecules and the maintenance of normal intestinal epithelial architecture and function come from the experiments of Hermiston and Gordon.^{41–43} They generated chimeric-transgenic mice in which islets of enterocytes transfected with a dominant negative N-cadherin mutant lacking the extracellular domain was found alongside normal intestinal mucosa. Expression of the construct in the villus enterocytes alone resulted in disrupted cell-cell and cell-matrix interactions, increased cell migration along the crypt-villus axis, loss of cellular differentiation and polarization, and increased apoptosis.⁴¹ When the N-cadherin null mutant was expressed along the entire crypt-villus axis, the consequent perturbation of cadherin-mediated cell-cell adhesion in the crypt epithelial cells resulted in an altered cell cycle leading to adenoma formation and perturbed mucosal barrier function and induced progressive inflammatory changes with features of Crohn's disease such as lymphoid aggregates, cryptitis, and crypt abscesses.⁴² Forced expression of E-cadherin in the same model resulted in decreased cell proliferation and migration and increased cell apoptosis in the upper part of the intestinal crypt.⁴³

Adhesion Molecules during Cell Migration

Cell motility is a complex process requiring dynamic and coordinated interactions between cells, their extracellular environment, and the cytoskeletal network. Distinct spatial and temporal changes in cell-cell and cell-matrix interactions occur not only during embryogenesis and differentiation but also during cell migration.^{3,44,45} It appears that the transition from a stationary to a motile phenotype requires perturbation of the E-cadherin-catenin complex and modulation of the expression, affinity, and binding specificity of integrins.⁴⁶

The initiation and progression of epithelial cell migration require the release of the adhesive tractions between epithelial cells, thought to occur through disruption of E-cadherin-mediated adhesion. As mentioned above, the stoichiometric balance underlying the various catenin pools and their complexes is critical for cell-cell adhesion. It is conceivable that a shift toward β -catenin-APC complexing and decreased β -catenin-E-cadherin binding may lead to perturbed E-cadherin-mediated adhesion and the initiation of cell migration.⁵ Interestingly, APC appears to play a functional role in cell motility, because it localizes *in vivo* to the end of cell processes and at the tips of microtubule bundles^{47,48} and because its forced expression in transgenic mice leads to a markedly disordered nonadhesive migratory phenotype.⁴⁹

β -Catenin and α -catenin also have been shown to bind the actin bundling proteins fascin⁵⁰ and α -actinin,⁵¹ respectively. The assembly and organization of actin bundles and networks are necessary for the extension of the lamellipodia and filopodia. The extending cellular processes go on to bind the substratum through integrin receptors at the leading edge of the migrating cell and the establishment of such stable cell-substratum adhesion generates traction and movement forward. This is

followed by release of adhesive contacts at the trailing edge of the cell.^{3,44,45} We have shown that $\alpha_2\beta_1$ -integrin receptor mediates the migration of colon carcinoma cells on collagen.⁵²

Cell Migration and Epithelial Restitution

Increased cell motility and migration are fundamental processes during the early reparative response of epithelial restitution in mucosal healing. This reparative mechanism is commonly seen following superficial mucosal ulceration in inflammatory bowel disease, such as ulcerative colitis and Crohn's disease. It consists of viable epithelial cells traveling rapidly from the ulcer margins over the denuded basement membrane to cover the defect and restore the structural and functional integrity of the gastrointestinal mucosa.^{5,46,53} During the initial response, there appears to be little or no cell proliferation and cell migration is the essential factor contributing to mucosal repair. Subsequently, however, cell proliferation and differentiation occur to re-establish normal tissue architecture and function.

We have shown that perturbation of E-cadherin-catenin mediated cell-cell adhesion is associated with cell migration during epithelial restitution. Using a wounded monolayer of a colorectal carcinoma cell line as an *in vitro* model of epithelial injury, migrating cells showed reduced membranous E-cadherin expression with abnormal cytoplasmic immunoreactivity.⁵⁴ Cytoplasmic E-cadherin is likely to reflect a nonfunctional molecule, as E-cadherin needs to be expressed on the membrane to mediate cell-cell adhesion. *In vivo*, using immunohistochemical and *in situ* hybridization techniques, we analyzed the expression and cellular localization of the E-cadherin-catenin complex in Crohn's disease, ulcerative colitis, and peptic ulcers and confirmed that the regenerating epithelium over the ulcer bases shows altered cellular localization and decreased levels of E-cadherin, α -catenin, and p120 expression.^{54,55} Such decreases in expression seemed to correlate with increased disease activity. Altered adhesion molecule expression and subcellular localization seem to be common phenomena in a variety of gastrointestinal disease states.

Growth Motility Factors and Epithelial Restitution

Several soluble growth factors and cytokines, such as the epidermal growth factor (EGF), the transforming growth factors α (TGF- α) and β (TGF- β), the hepatocyte growth factor/scatter factor (HGF/SF), and the newly described trefoil peptides, have been found to act as motogens promoting migration of epithelial cells and thus to play a role during epithelial restitution and mucosal repair (Figure 1).^{5,46} Recent data suggest that the stimulatory effect of these factors on epithelial restitution and regeneration requires modulation of cell-cell and cell-matrix interactions.

EGF is one of the most widely studied peptides involved in the maintenance of mucosal integrity. It is produced in the salivary glands and duodenal Brunner's glands and is highly homologous to TGF- α , which is expressed throughout normal gastrointestinal mucosa. Both of these peptides are able to bind EGFR and both have many similar biological activities relevant to gastrointestinal physiology.^{46,56} They both inhibit gastric acid secretion *in vitro* and *in vivo*, stimulate mucosal growth, and protect against the development of experimentally induced gastric ulcerations. During ulceration there is an increase in EGF and TGF- α expression at the periphery of the ulcer suggesting their role in mucosal healing.^{46,56} We and other groups have shown that both EGF and TGF- α stimulate migration *in vitro* of colonic epithelial cells on laminin and collagen and that both growth factors up-regulate the functional activity of at least two integrin molecules ($\alpha_2\beta_1$ and $\alpha_3\beta_1$) that are receptors for laminin and collagen.^{57,58} Such migration can be inhibited by monoclonal antibodies to the α_2 - and β_1 -integrin chains. Interestingly, when cells are promoted to a migratory state by the soluble factors TGF- α or TFF2 (discussed below), there is an increased binding of $\alpha_2\beta_1$ -integrin to the E-cadherin-catenin complex via β -catenin.⁵⁹ This cross-talk between integrins and cadherin leads to the unstable cell-matrix binding required to promote and direct cell migration.

We and others have reported that EGF as well as HGF/SF can induce a rapid tyrosine phosphorylation of β -catenin, γ -catenin, and p120 in colon carcinoma cells.⁶⁰ This is associated with a redistribution of E-cadherin from the intercellular junction to the apical surface of the cell membrane, resulting in increased cell dissociation and motility.⁶⁰ Although HGF/SF is produced primarily by cells of mesenchymal origin, it seems to promote epithelial cell motility and scattering on various extracellular matrix substrates.⁶¹ *In vivo*, HGF/SF mRNA and protein have been localized in the fibroblasts around gastric ulcers but not in gastric epithelial cells, suggesting that it acts in a paracrine fashion.⁶² *In vitro*, HGF/SF has a potent proliferative effect on gastric epithelial cells in addition to promoting cell migration and epithelial restitution. Such actions can be reversed by the administration of anti-HGF/SF antibodies.⁶²

The trefoil peptides are a highly conserved family of small, stable, secreted molecules in which the disulfide bonds between cysteine residues exhibit a common trefoil motif.⁶³⁻⁶⁵ The level of evolutionary conservation of the trefoil configuration suggests potentially important and similar functions. Three trefoil proteins have been identified in humans to date: pS2 (TFF1), human spasmodic polypeptide (TFF2), and intestinal trefoil factor (TFF3). Although the peptides have been found in a number of tissues including the brain, uterus, and breast, their predominant site of normal expression is the gastrointestinal tract.⁶³⁻⁶⁵ Within the gut, they seem to be found in a regionally specific pattern, particularly in association with mucins, suggesting a role in maintaining the integrity of the mucosal layer. Throughout the gastric mucosa, TFF1 is expressed in the mucus cells of the gastric pits, and TFF2 is expressed by the foveolar epithelial cells.

Both TFF1 and TFF2 are found in secreted mucus, and TFF2 mRNA is also expressed at the bottom of the antral and pyloric gastric glands.⁶⁶⁻⁶⁸ It is interesting to note that in TFF1 knockout mice loss of TFF1 expression results in loss of mucus production.⁶⁹ TFF3, on the other hand, is mainly found in the goblet cells of the small and large intestinal mucosa.^{70,71}

The trefoil peptides are ectopically overexpressed in a number of human cancers⁶⁵ and within gastrointestinal mucosa affected by chronic ulcerative and inflammatory lesions such as in Crohn's disease, ulcerative colitis, and gastroduodenal peptic ulceration, suggesting a role in mucosal healing and regeneration.⁷²⁻⁷⁵ They have also been found in the ulcer-associated cell lineage.^{65,70} *In vivo*, both TFF2 and TFF3 provide sufficient protection and reduce the extent and severity of ethanol- and indomethacin-induced acute gastric injury in rats.^{76,77} Furthermore, after a cryoprobe-induced gastric ulceration, rat TFF2 expression increased in the ulcerated area as early as 30 minutes after the insult, followed 2 days later by an ectopic expression of rat TFF3.⁷⁸ In fact, transgenic mice that overexpress TFF1 have increased resistance to intestinal damage⁷⁹ and TFF3 knockout mice have impaired mucosal healing and an expanded proliferative compartment in their intestinal epithelium.⁸⁰

All of the trefoil proteins have been shown to promote cell motility but, unlike many other motogenic factors, not cell division.⁴⁶ TFF1 transfection into a mouse adenocarcinoma cell line resulted in an enhanced dispersed growth pattern.⁸¹ Indeed, *in vitro* studies have shown that both recombinant TFF3 and recombinant TFF2 stimulate the migration of intestinal epithelial cells, promote wound healing, and lead to altered E-cadherin expression and cellular localization.^{76,82} They have also been found to be irreversibly cross-linked to specific binding sites that are present within gastric, colonic, and jejunal glands.^{83,84} However, the putative trefoil peptide receptors have yet to be cloned and characterized.

We have recently shown that TFF3 can induce a rapid tyrosine phosphorylation of β -catenin, γ -catenin, and the EGF receptor, reduce cell-cell and cell-substratum adhesion, and promote a migratory phenotype.⁸⁵ These responses were further associated with altered expression of the components of the catenin complexes, translocation of APC from the cytoplasm into the nucleus, and the induction of apoptotic changes.⁸⁶ Such observations are consistent with the suggestion that one of the functions of APC may be the regulation of cell shedding into the lumen at the top of the intestinal crypt.

It is interesting to note that tyrphostin, a competitive inhibitor of protein tyrosine kinases, inhibited the effects induced by TFF3 and re-established normal cell interactions *in vitro*,⁸⁶ confirming that tyrosine phosphorylation is an important mechanism by which TFF3-mediated changes are regulated. Indeed, we and others have shown that components of the catenin complexes can associate with molecules which either express tyrosine kinase activity or act as substrates for various receptor tyrosine kinases, such as EGFR and p120.^{17,85,87} Although tyrosine phosphorylation of β -catenin seems to be a common mechanism^{60,85,88} by which growth motility

factors modulate adhesion complexes and promote cell migration necessary for epithelial restitution, we detected no significant inhibition of catenin tyrosine phosphorylation by tyrphostin in our study.⁸⁶ Therefore, tyrosine phosphorylation of other molecules, such as EGFR or the putative trefoil peptide receptor, may play the important role in mediating the effects of TFF3. Interestingly, EGF has been shown to potentiate the effects of TFF3.⁸⁹ Such synergism suggests their complementary roles and linked receptor signaling pathways, possibly through β -catenin. Hirohashi, in this issue,⁷ further highlights the importance of catenin phosphorylation in the context of cancer.

Summary and Conclusions

Cell-cell and cell-matrix interactions are critical to the dynamic processes necessary for tissue morphogenesis in embryos and to the maintenance of complex differentiated tissues in adult organisms. The E-cadherin-catenin and integrin complexes influence and coordinate a variety of cellular processes including adhesion, differentiation, polarity, migration, proliferation, and survival. The suggestion of molecular cross-talk between the cadherin- and integrin-mediated cell adhesion systems, as well as the catenin and EGFR signaling pathways, during the regulation of such cellular processes has important implications for the understanding of gastrointestinal epithelial cell biology. It is therefore not surprising that adhesion-associated molecules are common targets for motogenic and mitogenic factors. The modulation or perturbation of cadherin, catenin, and integrin expression and function play a central role in gastrointestinal epithelial homeostasis and in various pathophysiological situations such as the repair of mucosal injury by epithelial restitution. This implies the potential for the clinical and pharmacological management of chronic ulcerative and inflammatory lesions through the manipulation of cellular adhesive mechanisms. However, to validate such an approach we need appropriate animal models which more closely mimic human gastrointestinal disease. Further development and refinement of such models combined with the elucidation of the biochemical and genetic processes involved will allow investigation into novel treatment, management, and prevention protocols.

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