Reviews

Cadherin adhesion in the intestinal crypt regulates morphogenesis, mitogenesis, motogenesis, and metaplasia formation

I Perry, R Hardy, C Tselepis, J A Jankowski

Abstract

The topographical organisation of the epithelium lining mucous membranes has been an intense point of research. One of the fundamental biological issues underpinning this and associated issues relates to the role and regulation of epithelial adhesion molecules. Adhesion between individual cells allows an intact layer to be formed, which is selectively permeable. In addition, the orchestrated regulation of multiple adhesion molecules allows the gradual transition from basal secretory cells to apical absorptive cells in the crypt-villus gradient. Moreover, it is becoming clear that no one class of adhesion molecule can sufficiently govern crypt architecture; however, the main cell-cell adhesion molecules are the cadherins and the related desmosomal cadherins. These latter molecules interact with the catenins, which bind directly or indirectly with cytoskeletal molecules such as Rho and Rac. In addition, other complex glycoproteins, such as the carcinoembryonic antigens, might contribute to adhesion, although their mechanisms of function are distinctly different. Integrins on the basal aspect of the cells also signal important morphoregulatory signals as a result of their binding to the extracellular matrix. The disruption of these physiological processes also provides a necessary and, in some cases, sufficient molecular mechanism for cancer invasion and metastasis, such as occurs in E-cadherin mutation positive familial gastric cancer.

(*J Clin Pathol: Mol Pathol* 1999;52:166-168)

Keywords: adenomatous polyposis coli; cadherin; cancer; catenin; crypt; metaplasia

The stroma of the intestine contains many cells but, in particular, the inflammatory cell infiltrate contributes to the mucosal architecture, even in phenotypically normal mucosa. These neutrophils and lymphocytes express a complex repertoire of adhesion molecules, which allows long distance homing and specific paracellular migration in the epithelium.

Thus, the formation and homeostasis of the epithelium requires a complex but inter-related array of adhesion molecules.

Introduction to adhesion molecules

The major cellular adhesion molecules can be classified according to structure into four general classes: cadherins, the immunoglobulin family, the integrin family, and selectins (glycoproteins and lectins). Migration of epithelial cells from the crypt bases to the superficial epithelial surface involves the constant formation and disruption of cell-cell and cell-stromal adhesive interactions, which therefore also have an important role in the maintenance of both cellular differentiation and the structural integrity of the human intestine.

Life, death, migration, and clonal expansion in the small intestine: the functional unit is the crypt-villus unit

In the adult small intestine, decisions affecting the proliferative status, lineage allocation, migration, differentiation, and death are made continuously and are executed rapidly along the crypt-villus axis. The entire crypt turnover is extremely rapid and enterocytes have a three day life span.¹ Stem cells at the base of the crypt divide to produce mainly long lived mucous and columnar progenitor cells, which in turn produce shorter lived transit amplifying cells in the midcrypt, capable of rapid upward migration.² Cells enter terminal differentiation or undergo apoptosis in the villus. It is currently thought that when the proliferative activity of a crypt passes a certain threshold the crypt is induced to bifurcate into two, starting at the base.³ Ultimately, these crypts, together with neighbouring identical crypts, divide again, producing a large contiguous group of epithelial cells with a common genotype, known as a "clonal patch", which is ~ 4 mm in size.³

Cadherins regulate adhesion, migration, and apoptosis of the small intestine in health and disease

BIOLOGICAL ROLES OF CADHERINS IN THE INTESTINE

Cadherins are a family of calcium dependent cell-cell adhesion molecules essential to the

Department of
Medicine and CRC
Institute for Cancer
Studies, University
Hospital,
Birmingham, B15
2TH, UK
I Perry
R Hardy
C Tselepis
J A Jankowski

Correspondence to:
Dr Jankowski.

Accepted for publication
27 May 1999

maintenance of intercellular connections at the adherens junctions.⁴⁻⁶ Adhesion occurs via five conserved "cadherin" domains in the extracellular portion, but an internal conserved catenin binding domain binds to the cytoskeleton.⁷⁻⁸ Cadherins also act as master morphoregulatory molecules that modulate epithelial polarity, plasticity, and survival during epithelial remodelling.⁹

EVIDENCE FOR CADHERIN BIOLOGY MODULATING PHENOTYPE IN VIVO

One fundamental question about the crypt-villus unit is whether epithelial cells are invested with complete responsibility for the control of their proliferation, migration, differentiation, and death programmes. Murine models that overproduce (forced) wild-type E-cadherin in the crypt of the small intestine have slowed proliferation and migration. Conversely, a chimaeric/transgenic murine model producing a dominant negative N/E-cadherin results in a Crohn's disease phenotype (P-cadherin alterations have not been studied),¹⁰ with increased proliferation and cell death as well as immature and uncoordinated differentiation signals. Chromosome 16, where E-cadherin and P-cadherin genes are located, is a major susceptibility locus for Crohn's disease in humans.¹¹ The nature of this inherited predisposition is unclear, but relatives of patients with Crohn's disease have an increased sensitivity to acetylsalicylic acid provoked permeability of the mucosal barrier compared with controls.¹² The increased permeability is thought to be an early feature of the disease rather than a result of the inflammatory process itself.¹³ Furthermore, we have data from human Crohn's disease *in vivo* indicating that the E-cadherin gene is strongly expressed in normal colorectal epithelium, whereas in Crohn's disease it is downregulated.¹⁴ In contrast, the gene encoding P-cadherin is dramatically upregulated in the ileocaecal region adjacent to fissures in Crohn's disease, whereas it is not normally expressed in this region.¹⁴ Comparisons of the presence of cadherins in Crohn's disease compared with other forms of colitis indicate there are no significant changes in the expression of cadherin in infective colitis.¹⁴ Second, ulcerative colitis is typified by downregulation of E-cadherin on the superficial compartment only, and such downregulation does not usually occur in the deep crypts. In addition, P-cadherin is only upregulated in severely ulcerated areas and then only in the superficial compartment.¹⁴

THE MECHANISM OF DIFFERENTIAL CADHERIN EXPRESSION IN CROHN'S DISEASE

The cadherin genes are tandemly linked on chromosome 16q22; however, evidence about a locus control region is unavailable, although the promoters have been analysed.¹⁵⁻¹⁶ Tumour necrosis α (TNF- α) decreases E-cadherin expression in small intestinal cells (I Perry *et al.*, 1999, unpublished) and may also increase P-cadherin selectively in inflamed epithelial cells, such as those found in the prostate.¹⁷ There are two phases to E-cadherin down-

regulation: early on, as a result of the disassembly of E-cadherin complexes via Rho and Rac GTPases, and, later on, as a result of transcriptional inhibition.¹⁸ We postulate that alterations to 16q in Crohn's disease, combined with local increases in TNF- α expression in the ileum, will decrease E-cadherin and increase P-cadherin expression in the ileum. Consequently crypt-villus units expressing low amounts of E-cadherin will be more susceptible to damage (fissures), whereas P-cadherin expressing clones will survive (skip lesions).

Alterations in cadherin biology might regulate changes in epithelial biology as a result of increased catenin regulated transcription and signalling via novel interactions

Catenins are members of the "armadillo" superfamily with distinct but overlapping functional domains.¹⁹ The cytoplasmic catenin pool is also regulated by the adenomatous polyposis coli (APC) tumour suppressor protein (perhaps also the APC2 gene product),²⁰⁻²¹ and glycogen synthase kinase (GSK3 β). The formation of this macromolecular complex regulates catenin degradation by the ubiquitin pathway²² and can be inhibited by Wnt signalling.²³⁻²⁴ Recently, along with others, we have identified germline E-cadherin mutations in families susceptible to gastric cancer.²⁵ These individuals have an increased frequency of intestinal metaplasia in unaffected areas; however, the adenocarcinomas are almost always of the diffuse type (rather than the intestinal type). It is believed that E-cadherin mutations result in increased catenin regulated transcription. Increased tyrosine phosphorylation of catenins may also increase cytoplasmic pools. Increased expression of β -catenin in mice will increase E-cadherin expression and perhaps other cadherins in an attempt to counteract the signalling activity of β -catenin. In addition, migration is also slowed, perhaps as a result of saturation of APC molecules, which are required to organise microtubules.²⁰ In compartments where increased β -catenin-E-cadherin complexes are present, enhanced apoptosis is also seen. Conversely when β -catenin is increased alone, especially in the crypt cells, increased proliferation occurs, perhaps as a result of low level stimulation of high motility group box transcription factors, such as Tcf-4. It is current theory that different cadherins may, in part, regulate this process as a result of differential affinities with catenins (JA Jankowski, 1996, unpublished) and the generation of alternative intracellular signals.

The internal domain of E-cadherin can also bind phosphatidylinositol 3 kinase (PI3K) in the absence of catenin binding (M Wakelam, 1996, unpublished). PI3K is a family of lipid kinases which phosphorylates inositol at the 3' position, yielding phosphatidylinositol (3,4,5) trisphosphate. PI3K is a heterodimer consisting of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. PI3K is activated when the regulatory subunits (SH2 domains) bind specific phosphotyrosine containing mo-

tifs on activated tyrosine kinase receptors. Current data suggest that PI3K activation at the adherens junctions might coordinate many cellular processes including adhesion, cytoskeletal rearrangement, and proliferation²⁶ during the cells upward migration in the crypt.

Association of inflammation with persistence of metaplasia: cytokines alter epithelial migration and catenin regulated transcription

The close association between lymphocytic cells and metaplasia suggests that the presence of a lymphoid stroma may be a requirement for metaplastic growth in at least certain stages of epithelial development. There are many reports of previous inflammation being associated with the development of gastrointestinal metaplasia, including gastric carditis and intestinal metaplasia,^{10 27–28} and pancreatitis and islet cell metaplasia.²⁹ Inflammatory cells may regulate epithelial biology either directly, as a result of lymphocyte–epithelial interactions via CD40 or Fas membrane receptors, or indirectly as a result of secreted cytokines. In chronic inflammation involving Barrett's mucosa, many cytokines are produced both by the inflammatory cell infiltrate and the epithelium. Recently, we have found that TNF- α and interleukin 1 β (IL-1 β) decrease membranous expression of the cell–cell adhesion molecule E-cadherin and β -catenin.³⁰

In conclusion, the interactions of the many adhesion molecules in epithelial tissues are complex. Furthermore, the perturbation of these processes is sufficient and necessary to explain altered epithelial structure and biology in inflammation and cancer.

This work was funded by the Cancer Research Campaign, UK.

- 1 Potten CS. Stem cells in gastrointestinal epithelium: numbers, characteristics and death. *Philos Trans R Soc Lond B Biol Sci* 1998;353:821–30.
- 2 Bjerknes M, Cheng H. Clonal analysis of mouse intestinal epithelial progenitors. *Gastroenterology* 1999;116:7–14.
- 3 Novelli MR, Williamson JA, Tomlinson IP, et al. Polyclonal origin of colonic adenomas in an XO/XY patient with FAP. *Science* 1996;272:1187–90.
- 4 Hermiston ML, Wong MH, Gordon JI. Forced expression of E-cadherin in the mouse intestinal epithelium slows cell migration and provides evidence for nonautonomous regulation of cell fate in a self-renewing system. *Genes Dev* 1996;10:985–96.
- 5 Yap AS, Niessen CM, Gumbiner BM. The juxtamembrane region of the cadherin cytoplasmic tail supports lateral clustering, adhesive strengthening, and interaction with p120ctn. *J Cell Sci* 1998;141:779–89.
- 6 Kuroda S, Fukata M, Nakagawa M, et al. Role of IQGAP1, a target of the small GTPases Cdc42 and Rac1, in regulation of E-cadherin-mediated cell–cell adhesion. *Science* 1998;281:832–5.
- 7 Braga VMM, Del Maschio A, Machesky L, et al. Regulation of cadherin function by rho and rac: modulation by

- junction maturation and cellular context. *Mol Biol Cell* 1999;10:9–22.
- 8 Danjo Y, Gipson IK. Actin “purse string” filaments are anchored by E-cadherin-mediated adherens junctions at the leading edge of the epithelial wound, providing coordinated cell movement. *J Cell Sci* 1998;111:3323–32.
- 9 Oberg S, Peters JH, DeMeester TR, et al. Inflammation and specialised intestinal metaplasia of the cardiac mucosa is a manifestation of gastroesophageal reflux disease. *Ann Surg* 1997;226:522–30.
- 10 Hermiston ML, Gordon JI. Inflammatory bowel disease and adenomas in mice expressing a dominant negative N-cadherin. *Science* 1995;270:1203–7.
- 11 Hugot J, Puig P, Rousseau C, et al. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. *Nature* 1996;379:821–3.
- 12 Soderholm JD, Olaison G, Lindberg E, et al. Different intestinal permeability patterns in relatives and spouses of patients with Crohn's disease: an inherited defect in mucosal defence. *Gut* 1999;44:96–100.
- 13 Madara JL, Parkos C, Colgan S. The movement of solutes and cells across tight junctions. *Ann N Y Acad Sci* 1992;664:47–60.
- 14 Jankowski J, Bedford F, Boulton R, et al. Alterations in classical cadherins associated with progression in ulcerative and Crohn's colitis. *Lab Invest* 1998;78:1155–67.
- 15 Faraldo ML, Rodrigo I, Behrens J, et al. Analysis of the E-cadherin and P-cadherin promoters in murine keratinocyte cell lines from different stages of mouse skin carcinogenesis. *Mol Carcinog* 1997;20:33–47.
- 16 Jarrard DF, Paul R, van Bokhoven A, et al. P-cadherin is a basal cell specific epithelial marker that is not expressed in prostate cancer. *Clin Cancer Res* 1997;3:2121–38.
- 17 Sokoloff MH, Tso CL, Kaboo R, et al. In vitro modulation of tumor progression associated properties of hormone refractory prostate carcinoma cell lines by cytokines. *Cancer* 1996;77:1862–72.
- 18 Wojciak-Stothard B, Entwistle A, Garg R, et al. Regulation of TNF α induced reorganisation of the actin cytoskeleton and cell–cell junctions by Rho, Rac, and Cdc42 in human endothelial cells. *J Cell Physiol* 1998;176:150–65.
- 19 Hart MJ, de los Santos R, Albert IN, et al. Downregulation of beta-catenin by human axin and its association with APC tumor suppressor, beta-catenin and GSK3 β . *Curr Biol* 1998;8:573–81.
- 20 Wong MH, Rubinfeld B, Gordon JI. Effects of forced expression of an NH₂-terminal truncated β -catenin on mouse intestinal epithelial homeostasis. *J Cell Biol* 1998;141:765–77.
- 21 van Es JH, Kirkpatrick C, van de Wetering M, et al. Identification of APC2, a homologue of the adenomatous polyposis coli tumour suppressor. *Curr Biol* 1999;9:105–8.
- 22 Korinek V, Barker N, Morin PJ, et al. Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC (–/–) colon carcinoma. *Science* 1997;275:1784–87.
- 23 Simcha I, Shuttman M, Salomon D, et al. Differential nuclear translocation and transactivation potential of b-catenin and plakoglobin. *J Cell Biol* 1998;141:1433–48.
- 24 Sadot E, Simcha I, Shuttman M, et al. Inhibition of b-catenin mediated transactivation by cadherin derivatives. *Proc Natl Acad Sci USA* 1998;95:15339–44.
- 25 Richards FM, McKee SA, Rajpar MH, et al. Germ-line E-cadherin gene (CDH1) mutations predispose to familial gastric and colorectal cancer. *Hum Mol Genet* 1999;4:607–10.
- 26 Scheid MP, Duronio V. Dissociation of cytokine-induced phosphorylation of Bad and activation of PKB/akt: involvement of MEK upstream of Bad phosphorylation. *Proc Natl Acad Sci USA* 1998;95:7439–44.
- 27 Goldblum JR, Vicari JJ, Falk GW, et al. Inflammation and intestinal metaplasia of the gastric cardia: the role of gastroesophageal reflux and H-pylori infection. *Gastroenterology* 1998;114:633–9.
- 28 Merrett MN. Ileal pouches: adaptation and inflammation. *Baillieres Clin Gastroenterol* 1997;11:175–93.
- 29 Lu L, Haber M. Pancreatic metaplasia in different disease states and the association with inflammation. *Lab Invest* 1997;76:336.
- 30 Perry I, Sanders DS, Eggo M, et al. Reduced cadherin/catenin expression in coeliac disease and in an in vitro model system. *Gastroenterology* 1999;116:G39993.