The role of the E-cadherin complex in gastrointestinal cell differentiation

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INTRODUCTION

The basic anatomical unit of the intestine is the crypt, a flask shaped structure lined by a monolayer of epithelium, which is known to be one of the fastest renewing cell populations of the body. Mitotic activity in the stem cells located at the base of the crypts provide a continuous supply of new cells. These cells subsequently embark on a differentiation pathway while migrating to the villus tip in the small intestine or mucosal surface in the colon, after which they undergo apoptosis and are shed into the lumen. This whole process is therefore continuously renewing, and can be influenced by a number of factors, for example, by the basement membrane on which the epithelium lies, by the pericryptal myofibroblasts that encase the crypt and by a host of other cellular components, mainly myofibroblasts and by extracellular matrix proteins present in the lamina propria, exerting their effects via cell-matrix adhesion molecules. There are also environmental factors that can influence the behaviour of the epithelium as well as hormones and various growth factors that play important roles in the maintenance of epithelial integrity. Over the past 10 years or so there has been much interest in the molecules mediating cell-cell interactions in the gut epithelium, and their role in the normal and abnormal development and differentiation of the gastrointestinal tract. It is these specialized macromolecular structures present at cell-cell junctions, that are essential for both intercellular adhesion and communication, that this review will focus on. Specifically E-cadherin, a cell-cell adhesion molecule, and its associated proteins, the catenins, and the regulatory role they play in the gastrointestinal tract including embryogenesis, cell migration, differentiation, repair of mucosal damage and neoplastic change will be discussed.

E-CADHERIN

E-cadherin is a 120-kDa transmembrane glycoprotein that forms a key component of adherens junctions. It is a calcium-dependant, homotypic intercellular adhesion molecule and is the predominant intercellular adhesion molecule expressed by intestinal epithelial cells (Takeichi 1991). It consists of an extracellular amino-terminal domain, a cell membrane spanning moiety, and a carboxy-terminal cytoplasmic domain (Kemler 1992). E-cadherin molecules form dimers at the basolateral cell surface which combine with other E-cadherin

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molecules on adjacent epithelial cells resulting in the formation of 'zipper' (Overduin *et al.* 1995; Shapiro *et al.* 1995). Normal E-cadherin expression and function is essential for the induction and maintenance of polarized and differentiated epithelia during embryonic development (Takeichi 1991). One of its functions appears to be the control of cell motility; during embryonic development there are spatial and temporal changes in E-cadherin expression which allow cell migration and morphogenesis (Takeichi 1988). 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 an early stage in embryogenesis (Larue *et al.* 1994; Riethmacher *et al.* 1995). Down-regulation of E-cadherin has been noted in the healing margins of ulcerated intestinal mucosa, which presumably allows regenerating epithelial cells to migrate over damaged areas (Dogon *et al.* 1995, Karayiannakis *et al.* 1998). Reduced or absent E-cadherin expression, determined by immunohistochemical methods, has been shown to be associated with a poorly differentiated phenotype, and infiltrative growth in oesophagus (Jankowski *et al.* 1994), stomach (Matsuura *et al.* 1992) and colon (Dorudi *et al.* 1993, Gagliardi *et al.* 1995). Studies using chimeric transgenic mice have further emphasized the importance of cadherin-mediated cell-cell adhesion in the intestinal epithelium (Hermiston & Gordon 1995a, Hermiston, Wong & Gordon 1996). In these experiments perturbation of E-cadherin expression in crypt cells resulted in increased cell migration along the crypt villus axis, increased cell proliferation and apoptosis and development of inflammatory changes of the type seen in inflammatory bowel diseases such as Crohn's disease. Forced expression of E-cadherin restored the normal sequence of events. In addition, disruption of E-cadherin-mediated cell adhesion in villous enterocytes resulted in loss of their differentiated polarized phenotype and alteration of their cell cycle (Hermiston & Gordon 1995b). Altered E-cadherin expression in inflammatory bowel disease has also been demonstrated (Hanby *et al.* 1996).

Adherens junctions only form on intercellular contact, leading to the notion that E-cadherin is a mediator of contact inhibition in cells (Cowin 1994). Additionally, E-cadherin binds to the actin cytoskeleton through α -catenin via β - and γ -catenin (see below) and when the actin filaments of the cytoskeleton polymerize, they release DNases, implicating E-cadherin in the control of apoptosis (Salomon & Diaz-Cano 1995).

E-CADHERIN-ASSOCIATED PROTEINS

The cytoplasmic domain of E-cadherin associates with groups of cytoplasmic proteins called catenins (α -, β - and γ -) and p120cas (Herrenknecht *et al.* 1991, McCrea & Gumbiner 1991, Nagafuchi, Takeichi & Tsukita 1991). A prerequisite for effective cadherin function is linkage to the actin cytoskeleton mediated by α -catenin via β - and γ -catenin and p120 cas. Deletion of the intracellular catenin-binding domain of E-cadherin or alterations in the functionally active catenins results in the loss of the ability of E-cadherins to enable cell adhesion, even if the extracellular binding domain is intact (Nagafuchi & Takeichi 1988, Hulsken, Birchmeier & Behrens 1994). β -catenin is the vertebrate homologue of the *Drosophila* segment polarity gene armadillo and is also involved in the wingless/wnt signalling pathway that controls developmental patterning in both insects and vertebrates (Pfeifer *et al.* 1991, McCrea *et al.* 1993, Miller & Moon 1996). Wnt1 is a secreted protein and the *wnt1* gene is the human homologue of *Drosophila* wingless (*wg*) gene. The main events during the wingless/wnt signalling pathway involve association of the Wg/Wnt family of secreted glycoproteins and their membrane receptor frizzled (Adler *et al.* 1990). This leads to phosphorylation and

activation of the dishevelled (Dsh) protein, which is recruited to the membrane area. This activated Dsh inhibits glycogen synthase 3b (GSK 3b/ZW-3), which normally phosphorylates β -catenin and directs it together with a complex involving adenomatous polyposis coli (APC), the gene product responsible for familial adenomatous polyposis, and the adaptor protein axin (conductin), to degradation by the ubiquitin-proteasome system (Aberle *et al* 1997). The net result is to maintain low levels of free cytoplasmic β -catenin. Alternatively, decreased degradation (in the absence of Wg/wnt signalling) leads to an increase in free cytosolic β -catenin which enters the nucleus and directly interacts with the high mobility group of transcription factors, lymphoid enhancer binding factor/T cell-specific factor (LEF/TCF), releasing them from their DNA binding site and leading to activation of gene expression (Behrens *et al* 1996, Barth *et al* 1997). Recently, the precise nuclear target of β -catenin has been demonstrated to be the transcription factor Tcf-4, which is essential for maintaining proliferating cells in intestinal crypts. Tcf gene deletions in transgenic mice cause intestinal hypoplasia (Korinek *et al* 1998). Therefore, proliferative activity in intestinal cells is regulated by the interaction of β -catenin with Tcf-4, and regulation of cytoplasmic levels of β -catenin directly modulates intestinal proliferation. One gene regulated by Tcf-4 is *c-MYC*. APC mutations in colorectal cancers cause increased β -catenin/Tcf-4 activity, leading to overexpression of *c-MYC* and promotion of neoplastic growth. Expression of dominant negative Tcf-4 in a colorectal cancer cell line with APC mutations significantly reduced endogenous levels of c-MYC protein and slowed tumour cell growth (He *et al* 1998). Therefore, mutations in APC lead to c-MYC stimulation of cell proliferation by preventing degradation of β -catenin.

The APC gene encodes a 310-kDa protein (Grodin *et al* 1991, Kinzler *et al* 1991) which has several structural domains, the middle section of the protein contains three successive 15-amino acid (aa) repeats, followed by seven related but distinct 20-aa repeats (Bodmer *et al* 1987). Both types of repeats are able to bind independently to β -catenin. A major function of APC, as discussed above, is to regulate the levels of free cytoplasmic β -catenin in the cell (Munemitsu *et al* 1995). APC and E-cadherin have in common the effect of decreasing the levels of cytoplasmic β -catenin and thereby stabilizing tissues. APC therefore competes directly with E-cadherin for binding to β -catenin and regulates β -catenin turnover inside the cell. Once APC mutation occurs, the equilibrium between these molecules is disturbed and this may be a major factor in the early development of colorectal tumours (Hinck *et al* 1994). It has been shown that in a significant proportion of sporadic colorectal human and MIN mice (mice with an APC germ-line mutation) (Moser *et al* 1990) adenomas, β -catenin is up-regulated compared to the adjacent nondysplastic epithelium. Additionally, β -catenin is abnormally localized in the nuclei of the dysplastic cells (Valizadeh *et al* 1997). Overexpression of β -catenin and its nuclear localization appear to be associated with mutations in the APC gene. Perhaps of potentially more significance are the recent data (Wasan *et al* 1998) suggesting there may be quantitative increases in β -catenin in normal (nondysplastic) heterozygote MIN mouse mucosa, suggesting that the deregulation process begins before any morphological change is visible.

In the developed world, colorectal cancer is the commonest non-smoking related cause of cancer deaths. Familial adenomatous polyposis (FAP) is an autosomal dominant disorder in humans characterized by the development of hundreds to thousands of colorectal polyps with invariable development of colonic carcinoma by middle age (Bodmer *et al* 1987). Germline mutations of the APC tumour-suppressor gene are responsible for FAP (Fearon & Vogelstein 1990) and although this accounts for less than 0.5% of all colorectal malignancies overall, it remains an excellent investigative model for sporadic disease, as mutations in APC are the

earliest genetic alteration in most sporadic colon carcinomas (Wasan *et al.* 1998). Consequently there has been much effort to elucidate its mechanism of action, and several lines of research have provided a basis for understanding the function of APC in regulation of cell proliferation and gene transcription. Most of the mutations in APC result in truncated APC protein which can complex with but not degrade β -catenin (Ilyas & Tomlinson 1997). The net result of APC mutation is therefore an increase in cellular β -catenin, which may trigger a cascade of events resulting in the initiation of adenomas. Moreover, when the truncated APC is bound to β -catenin it may not be available for incorporation into the E-cadherin/catenin complex. In this way APC mutation may also result in disrupted E-cadherin function. Thus it would appear that the first stages of adenoma development involve loss of regulation of β -catenin levels. Loss of normal tissue architecture may equate to some loss of normal control mechanisms such as signals from extracellular matrix via integrin receptors, resulting in abnormal growth (Pignatelli 1998). Indeed, early colorectal adenomas have increased levels of cytoplasmic β -catenin (Inomata *et al.* 1996).

So, mutated E-cadherin or APC genes produce proteins that are unable to effectively control β -catenin turnover, resulting in increased cytoplasmic levels of undegraded β -catenin. Increased β -catenin in the cytoplasm is transported to the nucleus and leads to increase c-MYC production and increased cellular proliferation. It is now clear that there are mechanisms other than direct cadherin engagement between cells that regulate this signalling system. The close linkage of certain receptor tyrosine kinases, such as epidermal growth factor receptor (EGF-R) to the cadherin complex is one pathway of regulation (Wilding *et al.* 1996, Efstathiou & Pignatelli 1998; Jawhari *et al.* 1999). Another is the finding that the trefoil factor TFF3 (intestinal trefoil factor), as well as epidermal growth factor (EGF), dynamically regulates the association of EGF-R and β -catenin with cadherin (Liu *et al.* 1997); this effect is at least partially mediated by the direct tyrosine phosphorylation of β -catenin in response to TFF3. In addition EGF as well as hepatocyte growth factor/scatter factor (HGF/SF) can induce a rapid tyrosine phosphorylation of β -catenin, γ -catenin and p120 in colon carcinoma cells (Shibamoto *et al.* 1994). This was 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. These results highlight similarities between TFF3 and other growth/motogenic factors such as EGF and HGF/SF, that enhance cell migration (or tumour invasion) by modulating intercellular adhesion (Efstathiou & Pignatelli 1998). Taken together these results in turn suggest that β -catenin represents a point of convergence between signalling cascades activated by either EGF or TFF, and acts as a common regulator of E-cadherin function. Given the complex and intimate interplay of all the above-mentioned factors, it is not surprising therefore that any perturbation in expression or function of any one of these molecules will result in the loss of intercellular adhesion and/or signalling to transcriptional control, leading to cell transformation and tumour progression.

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