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E-cadherin is required for intestinal morphogenesis in the mouse

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ABSTRACT

E-cadherin, the primary epithelial adherens junction protein, has been implicated as playing a critical role in nucleating formation of adherens junctions, tight junctions, and desmosomes. In addition to its role in maintaining structural tissue integrity, E-cadherin has also been suggested as an important modulator of cell signaling via interactions with its cytoplasmic binding partners, catenins, as well as with growth factor receptors. Therefore, we proposed that loss of E-cadherin from the developing mouse intestinal epithelium would disrupt intestinal epithelial morphogenesis and function. To test this hypothesis, we used a conditional knockout approach to eliminate E-cadherin specifically in the intestinal epithelium during embryonic development. We found that *E-cadherin* conditional knockout mice failed to survive, dying within the first 24 hours of birth. Examination of intestinal architecture at E18.5 demonstrated severe disruption to intestinal morphogenesis in animals lacking E-cadherin in the epithelium of the small intestine. We observed changes in epithelial cell shape as well as in the morphology of villi. Although junctional complexes were evident, junctions were abnormal, and barrier function was compromised in E-cadherin mutant intestine. We also identified changes in the epithelial cell populations present in *E-cadherin* conditional knockout animals. The number of proliferating cells was increased, whereas the number of enterocytes was decreased. Although Wnt/β-catenin target mRNAs were more abundant in mutants compared with controls, the amount of nuclear activated β-catenin protein was dramatically lower in mutants compared with controls. In summary, our data demonstrate that E-cadherin is essential for intestinal epithelial morphogenesis and homeostasis during embryonic development.

Introduction

As proper epithelial morphogenesis is essential for organ function, defining the roles that specific cell junction and adhesion molecules play in driving formation and maintenance of epithelial structure is necessary. Several studies of E-cadherin revealed an important role for this cell adhesion molecule in epithelial organization and maintenance (Perez-Moreno et al., 2003; Schneeberger and Lynch, 2004; Van Roy and Berx, 2008). For example, Madin–Darby canine kidney (MDCK) cells incubated with anti-E-cadherin antibodies failed to assemble not only adherens junctions but also tight junctions and desmosomes implicating E-cadherin as required for junctional complex formation (Gumbiner et al., 1988; Troxell et al., 2000). Studies of E-cadherin in vivo have been less clear in defining an essential role for E-cadherin in junctional complex assembly. Global knockout suggested that E-cadherin was required because the trophectoderm epithelium failed to form in its absence (Larue et al., 1994). Conditional ablation of E-cadherin from mammary epithelium, epidermis, thyroid follicular epithelium, and hepatic epithelium, however, did not result in tight junction or desmosome loss although epidermal deletion caused increased tight junction permeability and neonatal lethality because of a non-functional skin water barrier (Boussadia et al., 2002; Young et al., 2003; Tinkle et al., 2004; Tunggal et al., 2005; Battle et al., 2006; Cali et al., 2007). Studies looking at E-cadherin in the intestinal epithelium demonstrated a key role for E-cadherin in the maintenance of normal intestinal epithelial homeostasis (Hermiston and Gordon, 1995a, 1995b; Hermiston et al., 1996). Expression of a dominant-negative N-cadherin protein (NCADΔ) in villus enterocytes caused loss of endogenous E-cadherin protein resulting in cell adhesion and shape defects. Barrier function was also defective in NCADΔ-expressing enterocytes. Crypt cells that lacked NCADΔ protein and therefore maintained endogenous E-cadherin protein showed increased proliferation, which likely compensated for defective enterocytes on the villus (Hermiston and Gordon, 1995a). In contrast, over-expression of E-cadherin in mice resulted in slower cellular migration from crypt to villus, decreased proliferation, and increased apoptosis (Hermiston et al., 1996). Recently, Schneider et al. (2010) used tamoxifen-inducible Villin-Cre to remove E-cadherin from the adult mouse intestinal epithelium. Animals lacking E-cadherin developed

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hemorrhagic diarrhea requiring euthanasia. Epithelial architecture was abnormal with cells shedding into the lumen. There were changes in maturation and positioning of secretory lineages (goblet and Paneth cells). The proliferative zone was markedly expanded, and increased numbers of apoptotic cells were present. Migration of cells along the villus was also enhanced. Moreover, in contrast to deletion in other organ systems in which junctional complex assembly was unaffected by elimination of E-cadherin, loss of E-cadherin from the adult intestinal epithelium resulted in loss of both adherens junctions and desmosomes whereas tight junctions were unaffected (Schneider et al., 2010). The functionality of tight junctions, however, was not assessed.

Because E-cadherin has been implicated as playing critical roles in epithelial cell adhesion and signal transduction and because modulation of its expression in the adult small intestine caused epithelial defects, we proposed that loss of E-cadherin from the developing mouse intestinal epithelium would result in severe disruption of intestinal epithelial morphogenesis and homeostasis. Therefore, to assess the role that E-cadherin plays in intestinal development, we employed a conditional knockout approach using a non-inducible Villin-Cre, which directs robust recombination in the intestinal epithelium during development (Madison et al., 2002). We found that neonates lacking intestinal E-cadherin died shortly after birth. Villus structure and cell shape were both abnormal, and barrier function was compromised. We observed a decrease in the total number of epithelial cells present in mutant tissue. Of the specific differentiated cell types, enterocytes were lost whereas secretory populations were relatively stable. Proliferation was increased in animals with an E-cadherin deficient intestinal epithelium, and apoptosis was unchanged. Finally, β-catenin levels were decreased in mutant intestine compared with control. Paradoxically, we detected increased mRNA abundance of several β-catenin transcriptional targets in mutant epithelium compared with control epithelium. Based on these data, we conclude that intestinal E-cadherin expression is required for formation and maintenance of a functional intestinal epithelium in mice.

Materials and methods

Animals

Derivation of *Cdh1*^{loxP} (*Cdh1*^{tm2Kem}) and *Villin-Cre* (Tg(*Vil-cre*)997Gum) mice has been previously described (Boussadia et al., 2002; Madison et al., 2002). Embryonic mice were generated by timed matings considering noon on the day of a vaginal plug as E0.5. Genotypes were determined by PCR analysis of ear punch DNA following a standard protocol. PCR primers used were: *Cdh1*^{loxP}, gtgacaggaaaggcatatcagcaacaagat, gtgagctggtacccatggaggacactga; *Villin-Cre* caagcctggctcgacggcc, cgcgaacatcttcaggttct. For proliferation studies, 200 μg 5-ethynyl-2'deoxyuridine (EdU) was administered by intraperitoneal injection three hours prior to euthanizing animals. The Medical College of Wisconsin's Animal Care Committee approved all animal procedures used in this study.

Histochemistry, immunohistochemistry, and immunofluorescence

Tissue harvested from the midpoint of E18.5 small intestine was fixed in zinc formalin or 4% paraformaldehyde. Hematoxylin and eosin staining and alcian blue staining were performed according to standard procedures (Bancroft and Gamble, 2007). The Vector Red Phosphatase Substrate Kit (Vector Labs, Burlingame, CA) was used to detect alkaline phosphatase activity. For immunohistochemistry, antibodies were applied to tissue after citric acid antigen retrieval. To visualize staining, R.T.U. Vectastain Elite ABC reagent (Vector Labs, Burlingame, CA) and a Metal Enhanced DAB substrate kit (Thermo Scientific, Rockford, IL) were used. For immunofluorescence, fresh frozen sections were fixed with 3% paraformaldehyde prior to antibody staining. DAPI (Invitrogen, Carlsbad, CA; 1:5000) was used to visualize nuclei. EdU staining was performed using the Click-it Edu Alexa-Fluor 594 kit (Invitrogen, Carlsbad, CA). See Supplemental Table 1 for antibody details.

Electron microscopy

Embryonic (E18.5) small intestine was dissected into 2.5% glutaraldehyde in 0.1 M cacodylate buffer and embedded in EPON 812 epoxy resin. Sections (60 nm) were contrasted with uranyl acetate and lead citrate. For tracer experiments, embryonic mouse gut was dissected into sodium cacodylate buffer pH 7.4 and fixed in 2% glutaraldehyde containing either Lanthanum nitrate or Ruthenium red tracers (Lewis and Knight, 1992). Sections were examined using a Hitachi 600 transmission electron microscope.

Oligonucleotide array analysis

Total RNA (300 ng) isolated from three independent control and experimental E18.5 small intestines was used to prepare oligonucleotide array probes following the protocol described in the GeneChip Whole Transcript Sense Target Labeling Assay manual (Affymetrix, Santa Clara, CA). We hybridized a total of six Mouse Gene 1.0 ST arrays (Affymetrix, Santa Clara, CA), three for control samples and three for experimental samples, with fragmented, biotinylated ssDNA probes. Images were acquired using a GeneChip Scanner 3000 (Affymetrix, Santa Clara, CA). GeneChip Operating Software (GCOS) and NetAffx from Affymetrix, dChip 2010 software (Li and Wong, 2001), and Ingenuity Pathway Analysis software were used in combination to analyze the data. Log-transformed gene expression values were determined using dChip 2010. We selected a fold-change cutoff of $+/-2.0$ fold, $p \leq 0.05$. Differentially expressed transcript identifiers annotated using NetAffx are listed in Supplemental Table 2. The set of differentially expressed genes was analyzed by IPA for biological function analysis according to the method outlined in the Ingenuity 9.0 manual.

Epithelial cell isolation

Small intestine harvested from control and *E-cadherin* cKO E18.5 embryos was cut along its longitudinal axis and incubated in cell dissociation buffer (BD Biosciences, San Jose, CA) for 6 h at 4 °C with gentle agitation to release epithelial cells (Madison et al., 2005; Li et al., 2007).

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

DNase treated total RNA isolated from epithelial cells of three independent control and E-cadherin mutant E18.5 intestines was used to generate cDNA with the Superscript VILO cDNA synthesis kit (Invitrogen, Carlsbad, CA). qRT-PCR was performed using TaqMan assays and TaqMan Gene Expression Master Mix (Applied Biosystems, Carlsbad, CA). TaqMan assays utilized are listed in Supplemental Table 3. Data were analyzed using DataAssist software (Applied Biosystems, Carlsbad, CA). *Gapdh* was used for normalization. Each gene was assayed in at least two independent experiments. Error bars represent standard error of the mean (SEM).