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Permalink
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Journal
The Journal of cell biology, 129(2)

ISSN
1540-8140

Authors
Hermiston, ML
Gordon, JI

Publication Date
1995-04-01

DOI
10.1083/jcb.129.2.489

Peer reviewed
In Vivo Analysis of Cadherin Function in the Mouse Intestinal Epithelium: Essential Roles in Adhesion, Maintenance of Differentiation, and Regulation of Programmed Cell Death

Michelle L. Hermiston and Jeffrey I. Gordon
Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, Missouri 63110

Abstract. A model system is described for defining the physiologic functions of mammalian cadherins in vivo. 129/Sv embryonic stem (ES) cells, stably transfected with a dominant negative N-cadherin mutant (NCADA) under the control of a promoter that only functions in postmitotic enterocytes during their rapid, orderly, and continuous migration up small intestinal villi, were introduced into normal C57B1/6 (B6) blastocysts. In adult B6×129/Sv chimeric mice, each villus receives the cellular output of several surrounding monoclonal crypts. A polyclonal villus located at the boundary of 129/Sv- and B6-derived intestinal epithelium contains vertical coherent bands of NCADA-producing enterocytes plus adjacent bands of normal B6-derived enterocytes. A comparison of the biological properties of these cell populations established that NCADA disrupts cell-cell and cell-matrix contacts, increases the rate of migration of enterocytes along the crypt-villus axis, results in a loss of their differentiated polarized phenotype, and produces precocious entry into a death program. These data indicate that enterocytic cadherins are critical cell survival factors that actively maintain intestinal epithelial function in vivo.

The cadherins are a superfamily of transmembrane glycoproteins that mediate homophilic, Ca2+–dependent interactions between cells (Takeichi, 1991; Kemler, 1993). E-cadherin is the predominant cadherin found in epithelial cells. Studies in cultured cells suggest that E-cadherin may play a critical role in establishing and/or maintaining a differentiated phenotype. For example, disrupting E-cadherin–mediated adhesion can lead to dissociation of epithelial monolayers and dedifferentiation to a fibroblast-like morphology (Behrens et al., 1985; Gumbiner and Simons, 1986). Expression of E-cadherin in nonepithelial cell lines can produce an adherent, polarized, epithelial-like monolayer (Nagafuchi et al., 1987; McNeill et al., 1990). E-cadherin can induce assembly of adherens and other types of junctional complexes (Gumbiner et al., 1988; Jongen et al., 1991; Watabe et al., 1994), maintain the organization of the actin cytoskeleton (Frixen and Nagamine, 1993), promote a polarized distribution of organelles and cellular proteins (McNeill et al., 1990), and inhibit cell proliferation (Watabe et al., 1994). Downregulation of E-cadherin expression in transformed cell lines is associated with dedifferentiation and acquisition of the capacity to invade, suggesting that cadherins may function as tumor suppressors in vivo (Behrens et al., 1989; Frixen et al., 1991; Vleminckx et al., 1991).

Members of the cadherin family have distinct spatial and temporal patterns of expression during embryonic development and in the adult (for review see Takeichi, 1988; Ranscht, 1994). There have only been a limited number of reports describing the results of manipulating cadherin function in vivo. Expression of dominant-negative cadherin mutants in Xenopus embryos disrupts cell adhesion and tissue morphogenesis (Kinter, 1992; Levine et al., 1994; Dufour et al., 1994; Holt et al., 1994). Mouse embryos homozygous for an E-cadherin null allele are unable to form a trophoeotdermal epithelium or a blastocyst cavity (Ikarashi et al., 1994). Because of this early embryonic lethality, it has not been possible to define, in vivo, the physiologic functions of mammalian cadherins either during or after completion of organogenesis.

The mouse intestinal epithelium provides an attractive model for analyzing cadherin function because the entire developmental sequence of cell proliferation, lineage allocation, migration-associated differentiation, and programmed cell death can be surveyed at any moment in time along its crypt-villus axis. Perpetual cellular renewal is fueled by multipotent stem cells located near the base of each crypt of Lieberkühn (Locstein et al., 1993). Studies of aggregation chimeras indicate that by the time intestinal morphogenesis is completed during the third postnatal week, each crypt is monoclonal, supplied by stem cells with identical genotypes.
and each villus is polyclonal, supplied by several surrounding crypts (Schmidt et al., 1983, 1988). The stem cells' immediate descendants undergo rapid amplification in the middle third of the crypt and are allocated to one of the four principal epithelial lineages. Differentiation is completed during a bipolar migration. Defensin- and growth factor-producing Paneth cells migrate downward to the base of the crypt where they reside for ~20 d before being removed by phagocytosis (Cheng, 1974; Bry et al., 1994). Absorptive enterocytes, enteroendocrine, and mucus-producing goblet cells migrate upward in vertical coherent bands from each crypt to the apical extrusion zone of a surrounding villus (Schmidt et al., 1985). The sequence of proliferation in cell cycle arrest, migration-associated differentiation, followed by programmed cell death and extrusion from the villus tip is repeated every 3–5 d for these three lineages (Wright and Irwin, 1982; Gavioli et al., 1992; Hall et al., 1994; for review see Hermiston et al., 1994). The stem cell hierarchy and functional organization of the intestine's crypt-villus units are ideally suited for creating chimeric-transgenic mouse models to study the effects of various gene products on epithelial cell biology (Hermiston et al., 1993). Embryonic stem (ES) cells (129/Sv origin), stably transfected with a recombinant DNA consisting of an intestine-specific promoter linked to a gene encoding the protein of interest, are introduced into a normal C57B1/6 (B6) blastocyst. A polyclonal villus positioned at the border of 129/Sv and B6 are introduced into a normal C57B1/6 (B6) blastocyst. A bryonic stem (ES) cells (129/Sv origin), stably transfected on epithelial cell biology (Hermiston et al., 1993). E-cadherin is the predominant cadherin in the intestinal epithelium, although distantly related family members are also detectable (Boller et al., 1985; Berndorff et al., 1994; Dantzig et al., 1994). Experiments in Xenopus embryos and a transfected mouse keratinocyte cell line have shown that an N-cadherin mutant lacking the extracellular domain (NCADA) can disrupt the functions of heterologous cadherins (Kinter, 1992; Fujimori and Takeichi, 1993). We expressed NCADA in enterocytes which account for >90% of the cells in the small intestinal epithelium (Cheng and Leblond, 1974). Expression was confined to villus-associated enterocytes so that we could study the contributions of cadherins to cell–cell and cell–matrix adhesion, to the regulation of cell migration rates and pathways, to the establishment and maintenance of a polarized differentiated state, and to the programming of cell death.

Materials and Methods

Construction of Transgenes

A 1.8-kb XhoI–KpnI DNA fragment, containing the neomycin resistance gene under the control of the phosphoglycerate kinase promoter (pgkNeo), was excised from pPNT (Tybulewicz et al., 1991) and subcloned into XhoI–KpnI-digested pBluescript I KS+ (Stratagene, La Jolla, CA), yielding pPgkNeoBS. A 3.5-kb EcoRI fragment containing nucleotides -1178 to +28 of rat Rabpl linked to nucleotides +3 to +2150 of the human growth hormone gene (I-FABP -178 to +28) hGH; Sweetser et al., 1988) was placed at the EcoRI site of pPkgNeoBS, generating pl178hGHpNeo with I-FABP -178 to +28 hGH located upstream and in the same transcriptional orientation as the pgkNeo selection cassette.

The human growth hormone gene contains a BamHI site at its nucleotide +3. pl178thGHDon has two additional BamHI sites, one located 3' to pgkNeo and the other in the pBluescript polylinker 5' to I-FABP -178 to +28 hGH. These two sites were eliminated by partial digestion of the plasmid.

Abbreviations used in this paper: B6, normal C57Bl/6; BrdU, 5'-bromo-2'-deoxyuridine; ES, embryonic stem; GMA, Glycine Max agglutinin; hGH, human growth hormone; NCADA, N-cadherin mutant lacking the extracellular domain; PAP, peroxidase-anti-peroxidase; PAS, Periodic Acid Schiff; TdT, terminal deoxynucleotidyl transferase; TUNEL, dUTP nick end labeling; UEA-I, Ulex europeaus agglutinin type 1.
with BamHI, treatment with Klenow, ligation, followed by one more cycle of this process, yielding pIl178hGHPneoAB, pS72NCADAc (kindly provided by Chris Kinter) contains a dominant-negative fragment at the downstream initiator ATG of hGH.}

end was filled in with Klenow, BglII linkers were added, and the 1.2-kb eDNA was excised from pSP72NCADAc with XhoI and EcoRV. The XhoI fragment provided by Chris Kinter contains a dominant-negative with BamHI, treatment with Klenow, ligation, followed by one more cycle after electroporation. 24 D3-Il178hGH colonies and 12 D3-Il178NCADA colonies in B-agarose (New England Biolabs, Beverly, MA). ES cells (2 × 10⁶/0.5 ml PBS) were electroporated with 10 μg of the purified DNA fragments using a Gene Pulser (960 μF, 200 nV, 0.4-cm cuvette). Cells were plated at a density of ∼2 × 10⁶ cells/60-mm feeder plate. G418 selection was begun 36 h after electroporation. 24 D3-Il178hGH colonies and 12 D3-Il178NCADA colonies were randomly selected from the 500-700 G418-resistant colonies obtained 10 d after transfection. They were expanded to a 48-well plate. After 2–3 d, confluent clones were harvested. Seventy-five percent of the cells were passaged to a 35-mm dish, grown to near-confluence, trypsinized, and frozen in freezing mix (10% DMSO, 40% FCS, and 50% ES cell media). The remaining cells were cultured for 4–5 d in gelatinized 24-well plates for subsequent isolation of genomic DNA.

**Characterization of D3-Il178hGH and D3-Il178NCADA ES Cell Lines**

Mouse intestinal fatty acid-binding protein is not detectable by Western blot analysis of lysates prepared from D3 ES cells maintained on Sto cells (Green, R., and J. Gordon, unpublished data). To determine if I-FABP-Il78 is silent in ES cells, medium harvested from each of the 24 confluent D3-Il178hGH cell lines were assayed in duplicate for the presence of hGH with a sensitive radioimmunoassay (Nichols Institute, San Juan Capistrano, CA). This radioimmunoassay does not recognize mouse or bovine growth hormone and can detect as little as 1 ng hGH/mL. No hGH was found in any of the samples.

Genomic DNA was purified from each stably transfected ES cell line and screened for the presence of hGH genomic sequences by PCR. Oligonucleotide primers to the antisense strand of exon I (5'-AGGTCrC,-CCTTTGACA-3') and to the sense strand of exon 3 (5'-CTCAGAGGGTGTTTCTCCTCCGTGGT-3') were used to amplify a 360-bp hGH fragment. The PCR mixture (final volume = 20 μl) contained 50 mM KCI, 10 mM Tris (pH 8.4), 2 mM MgCl2, 2 mg/ml gelatin, 200 μM dNTPs, 10 μM of each primer, 0.7 U AmpliTaq (Perkin Elmer/Cetus, Norwalk, CT), and 1 μg genomic DNA. The following cycling conditions were used: denaturation = 1 min at 94°, annealing = 1 min 30 s at 55°, and extension = 2 min at 72° for a total of 25 cycles. All G418-resistant clones surveyed contained hGH DNA.

**Production and Maintenance of Chimeric-Transgenic Mice**

Moruli were harvested from superovulated, 3-week-old B6 females that had been mated to B6 males. The moruli were cultured overnight at 37°C to the blastocyst stage under an atmosphere of 5% CO₂/95% air in Brinster's media (GIBCO BRL, Gaithersburg, MD) supplemented with penicillin and streptomycin. Two independent D3-Il178hGH cell lines were injected into the B6 blastocysts (10–20 ES cells/embryo) using standard methods (Bradley, 1987). Ten independent D3-Il178NCADA clones were injected (5–20 ES cells/embryo).

Animals were reared in microisolator cages under a strictly controlled light cycle (lights on at 0600 h and off at 1800 h). Mice were given a standard chow diet (Ralston Purina No. 5010) and water ad libitum. A sentinel screening program as well as serological tests of blood obtained by cardiac puncture at time of sacrifice of each chimeria verified that the chimeras were free of Hepatitis, Minute, Lymphocytic Choriomeningitis, Ectromelia, Polyoma, Sendai, K, Pneumonia, and MVA viruses.

**Lectin Staining of Wholemount Preparations of Small Intestine**

6–8-wk-old male and female B6*Il129/Sv, B6*Il129/Sv-Il178hGH, or B6*Il129/Sv-Il178NCADA male and female mice (n = 2–4 animals/ES cell line) were given an intraperitoneal injection of 5-bromo-2'-deoxyuridine (BrDU; Sigma; 120 mg/kg body weight) and 5-fluoro-2'-deoxyuridine (Sigma; 12 mg/kg) 60 h before sacrifice to label crypt cells in S-phase and monitor their subsequent migration along the crypt-villus axis. The entire gastrointestinal tract was removed en bloc immediately after sacrifice, flushed with ice-cold PBS, fixed in Bouin's solution for 5–12 h, and then washed with 70% ethanol. The small intestine was divided into equal thirds (designated duodenum, jejunum, and ileum). Each segment was opened with an incision along its cephalicaxial axis, and then rolled up from its proximal to distal end. Each of the resulting Swiss rolls (Griffiths et al., 1988) was cut in half, parallel to the cephalicaxial axis, placed in a tissue cassette with the cut edge on one-half facing down and the cut edge of the other half facing up, embedded in paraffin, and forty, 5-μm thick serial sections were cut. Every fifth section was stained with hematoxylin and eosin to define cellular morphology and crypt-villus architecture. Bouin's fixed samples of liver, kidney, spleen, stomach, colon, lung, heart, pancreas, skeletal muscle, skin, gonads, and brain, isolated from B6*Il178hGH D3 chimeras, were analyzed for the presence of immunoreactive hGH (see below). Small intestines from two mice/cell line were fixed in 10% phosphate buffered formalin and processed in the same fashion.

The methods used for single and multilabel immunocytochemical staining of paraffin-embedded sections are described in several publications (Roth et al., 1990; Hermiston et al., 1992; Falk et al., 1994). Briefly, sections were deparaffinized, and then rehydrated in PBS. Endogenous peroxidase activity was blocked by incubation in 1% H₂O₂/PBS if peroxidase-anti-peroxidase (PAP) development methods were used. An enzymatic unmasking step was included to visualize some antigen-antibody or lectin-glycoconjugate complexes. This entailed incubation of sections for 15 min at 37°C in a solution containing 1 mg/ml chymotrypsin (prepared in 7 mM CaCl₂, pH 7.8). Sections were then placed in PBS-blocking buffer (BSA 1%, w/v), powdered skin gel (0.2%, w/v), and 5% H₂O₂ (prepared in 7 mM CaCl₂, pH 7.8). The following primary antibodies were used: (a) anti-hGH (final dilution = 1:2,000; DAKO), goat anti-BrdU (1:3,000; Cohn and Lieberman, 1984), mouse anti-β-actin (1:1,000; Sigma), rabbit anti-rat liver fatty acid-binding protein (specificity = villus-associated enterocytes; final dilution = 1:10,000, Sweetser et al., 1998), rabbit anti-pan-cadherin (1:2,000, Sigma), rabbit anti-laminin (1:1,000, Sigma), rabbit anti-collagen IV (1:1,000, Collaborative Biomedical Products, Bedford, MA), and rabbit anti-heparin sulfate proteoglycan (1:200, Chemicon, Temecula, CA). A rat anti-E-cadherin monoclonal antibody (1:400; kindly provided by Rolf Klemm, Max Planck Institute; Vestweber and Klemm, 1985) was used on
Figure 2. Immunocytochemical analysis of cadherin accumulation along the crypt-villus axis in adult B6+/129/Sv and B6+129/Sv-Ile1178NCADA chimeras. (A-C) Sections from the proximal ileum of a normal B6+/129/Sv mouse (coat color chimerism = 95% 129/Sv). (A) Apex of a villus stained with a rat E-cadherin mAb (visualized with indocarbocyanine [Cy3]-conjugated donkey anti-rat Ig). The open arrows point to the apical junctional complexes. Note that immunoreactive protein is present throughout the lateral and basilar surfaces of enterocytes but is not detectable in their cytoplasm. (B) Lower power view of the entire crypt-villus axis. Arrows point to the lateral surfaces of crypt epithelial cells.
formalin-fixed tissues. Control experiments demonstrated that none of the secondary antibodies labeled tissue sections in the presence of preimmune sera or in the absence of primary antisera.

The following five lectins were employed, all at a final concentration of 5 μg/ml: peroxidase- or biotin-conjugated UEA-1 (Sigma; carbohydrate specificity = Fucα1-2Gal; GaINAcα3GalNAcβ3Galβ3GalNAcβ4Gal-), peroxidase-conjugated Dolichos biflorus agglutinin (Sigma; Galβ3GalNAcβ3Galβ3Gal), peroxidase-conjugated Helix pomatia agglutinin (Sigma, c-GalNAcβ3GalNAcβ3Galβ3GalNAcβ4Gal-) and peroxidase-conjugated Jacalin (Artocarpus integrifolia agglutinin, EY-Labs [San Mateo, CA], Galβ3GalNAcβ3Galβ3GalNAcβ4Gal), and peroxidase-conjugated soybean α-terminal agglutinin (Glycine max) agglutinin (Sigma, GalNacβ3GalNAcβ3GalNAcβ3Galβ3GalNAcβ4Gal). Molecular lectin lectin recognizes GalNacα3-glycoconjugates including the Tn antigen (GalNAcα3Galβ1-4GlcNAcβ1-4Glc) and its sialylated counterpart, sialyl-Tn (NeuAcα2,6Galβ1-3GalNAcβ1-4GlcNAcβ1-4Glc). The distributions of mouse E-cadherin along the crypt-villus axis parallels the proliferation of junctional complexes which occurs as enterocytes approach the villus tip (Madara, 1990).

Enterocyte levels of E-cadherin are equivalent in crypt-villus units located in the duodenum, jejunum, or ileum (data not shown). Multi-label studies with UEA-1 and the E-cadherin mAb revealed that 129/Sv- and B6-enteroocytes located at a given cell stratum of a polyclonal villus have similar intracellular concentrations and distributions of E-cadherin (data not shown).

**Nucleotides −1178 to +28 of the Rat Intestinal Fatty Acid-binding Protein Gene Can Deliver Foreign Gene Products to Villus-associated Enteroocytes in B6×129/Sv Mice**

Nucleotides −1178 to +28 of the rat intestinal fatty acid-binding protein gene (I-FABP−1178 to +28) have been used to express a variety of gene products in the small intestinal epithelium of transgenic mice (Cohn et al., 1992; Kim et al., 1993; Simonet et al., 1994; Zhou et al., 1994). I-FABP−1178 to +28 is activated on embryonic day 15, coincident with initial cytodifferentiation of the pseudotrophified intestinal endoderm to an epithelial monolayer overlying nascent villi. Expression is sustained at least through the first 18–24 mo of postnatal life (Kim et al., 1993). Reporter synthesis is confined to enterocytes, is only initiated as these cells exit the crypt, and is maintained until they are exfoliated at the villus tip (Cohn et al., 1992). Regional variations in I-FABP−1178 to +28 reporter expression are established and maintained along the duodenal-ileal axis with highest steady state levels occurring in the distal jejunum and proximal ileum (e.g., Cohn et al., 1992; Kim et al., 1993).

To assure ourselves that the expression domain of I-FABP−1178 to +28 is not affected by genetic background or by the pgkNeo selection cassette used to identify stably transfected ES cells, chimeric-transgenic mice were generated that contained this promoter linked to the human growth hormone (hGH) gene followed by pgkNeo. Wholomounts of small intestine, prepared from 6–8-wk-old B6×129/Sv-I178hGH mice, were stained with UEA-1 (n = 5 mice; 129/Sv coat color >80%). Highly chimeric areas (Fig. 3 A) were excised from the duodenum, jejunum, and ileum, and subjected to multi-label immunocytochemical analyses. The lineage-specific, differentiation-dependent, and cephalocaudal patterns of hGH accumulation were comparable to those obtained in multiple pedigrees of I178hGH mice produced by pronuclear injection: i.e., hGH is confined to ES-derived, villus-associated enterocytes and absent from crypts (Fig. 3 B).

**Results**

**E-cadherin Expression in Normal B6×129/Sv Mouse Intestine**

The distributions of mouse E-cadherin along the crypt-villus and duodenal-colonic axes have not been reported. Therefore, sections of small intestine were prepared from 6–8-wk-old B6×129/Sv mice that had been produced with nontransfected D3 ES cells (n = 5 animals; >95% 129/Sv contribution to coat color). Incubation of the sections with an E-cadherin monoclonal antibody (Westweber and Kemler, 1985) revealed intense punctate staining at apical junctional complexes (Fig. 2, A and B; cf Boller et al., 1985). Weaker staining occurs along the lateral surfaces of epithelial cells. Basilar surface staining is also apparent while cytoplasmic staining is absent. A pan-cadherin antiserum that recognizes a conserved epitope found at the COOH-terminal cytoplasmic tail of all known "classical" cadherins (Geiger et al., 1990; Kemler, 1992) gave similar results (Fig. 2 C). Both antisera disclosed that cadherins were associated with the apical rather than basolateral surfaces of crypt epithelial cells (Fig. 2 B). The intensity of staining increases at sites of cell--cell contact as enterocytes approach the villus' apical extrusion zone (Fig. 2, B and C). This distribution of E-cadherin along the crypt-villus axis parallels the proliferation of enterocytes, which is a conserved feature of cell stratification as enterocytes migrate up these villi (Madara, 1990).

Where E-cadherin is not detectable, in contrast to villus-associated enterocytes. (C) The section was incubated with a rabbit pan-cadherin antiserum and antigen-antibody complexes were visualized with Cy3-conjugated donkey anti–rabbit Ig. The cellular levels and distributions of cadherins are similar to what is observed with the E-cadherin mAb. (D–F) The intracellular level and distribution of cadherins are perturbed in villus-associated, 129/Sv-1178NCADA enterocytes. (D) Double exposure of a section of B6×129/Sv-I178NCADA ileum incubated with rat E-cadherin mAb (visualized with Cy3-labeled donkey anti–rat Ig) and biotin-conjugated UEA-1 (visualized with FITC-conjugated extra-avidin). A comparison of E-cadherin immunoreactivity (red) in UEA-1-positive enterocytes (stained green at their apical brush border) and UEA-1-negative enterocytes located at various cell strata along the crypt-villus axis (open arrows) reveals that expression of NCADA is associated with marked reductions in cellular E-cadherin levels. (E) Section of ileum stained as in D. The open arrows point to clumps of E-cadherin located at the basolateral surfaces of 129/Sv-I178NCADA enterocytes. The pattern is quite distinct from that encountered in the adjacent band of UEA-1-negative B6 enterocytes (closed arrows). (F) Ileal villi containing a wholly 129/ Sv-I178NCADA-derived population of enterocytes were sectioned parallel to their crypt-villus axis. Sections were incubated with rabbit pan-cadherin antiserum (visualized with Cy3-labeled donkey anti–rabbit Ig). As enterocytes migrate up these villi, there is a pronounced increase in steady state levels of immunoreactive cadherins at the surface of cells as well as throughout their cytoplasm (e.g., closed arrows). The open arrows highlight examples of cadherin aggregates that appear near the apex of enterocytes. Bars, 25 μm.
Figure 3. Immunocytochemical analysis of B6×129/Sv-Il178hGH mice. (A) Wholemount of the distal third of the intestine incubated with peroxidase-conjugated UEA-1 (visualized with 3',3'-diaminobenzidine). Clusters of wholly UEA-1-negative B6-derived villi (e.g., open arrow), wholly UEA-1-positive 129/Sv-Il178hGH-derived villi (closed arrowheads), and striped polyclonal supplied by both monoclonal B6- and monoclonal 129/Sv-Il178hGH crypts (closed arrows) are present. (B) A portion of the wholemount preparation shown in A, containing wholly UEA-1-positive villi, was embedded in paraffin and sectioned parallel to the crypt-villus axis. The sections were incubated with rabbit anti-hGH sera followed by gold-labeled goat anti-rabbit Ig and viewed with reflected light polarization microscopy. hGH is present in Golgi apparatus of villus-associated enterocytes (closed arrowheads). No immunoreactive protein is detectable in villus-associated goblet cells (closed arrows) or in the crypts (open arrows).

The Phenotype Produced by Expressing NCADA in Villus-associated Enterocytes

Evidence That High Percentage B6×129/Sv-Il178NCADA Chimeras Are Not Viable. I-FABP-~m°+28 was used to direct expression of the Xenopus dominant-negative N-cadherin mutant (Kinter, 1992). We initially injected six independent, cloned 129/Sv-Il178NCADA cell lines into B6 blastocysts (15-20 ES cells/blastocyst). Birthrates were low: only 28% of injected blasts produced live-born mice. Only 20% of the liveborn animals were chimeric as judged by their coat color (129/Sv contribution <40%). Cesarean section of late gestation recipient mothers revealed evidence of multiple resorbed fetuses. In contrast, injection of the nontransfected parental D3 ES cell line (or Il178hGH ES cells) resulted in threefold higher birth rates with >60% of these mice having >80% 129/Sv contribution to coat color. By reducing the number of ES cells injected/blastocyst to 5-10, we were able to produce B6×129/Sv-Il178NCADA mice from eight independent cell lines. All these animals appeared healthy and their weights at 6–8 wk were similar to those of comparably aged, comparably chimeric B6×129/Sv mice.

Analysis of Wholemount Preparations of B6×129/Sv-Il178NCADA Intestine. UEA-1 staining of wholemount preparations of small intestine from 6–8-wk-old normal B6×129/Sv mice revealed coherent columns of 129/Sv enterocytes extending from the crypt-villus junction to the apical extrusion zone of a polyclonal villus (Fig. 4, A and B). In 6–8-wk-old B6×129/Sv-Il178NCADA mice, UEA-1-positive 129/Sv-Il178NCADA enterocytes appeared to have defects in adhesion and migration. There were gaps between UEA-1-positive enterocytes within a given column. There were also focal clusters of bulbous and deformed cells that
Figure 5. B6**129/Sv-II178NCADA mice display a variety of histologic abnormalities in their villus-associated epithelium. (A) Hematoxylin- and eosin-stained section of villi located in the proximal ileum of a normal B6**129/Sv mouse (95% 129/Sv coat color). (B-E) Hematoxylin-and eosin-stained sections of small intestine from 6-wk-old B6**129/Sv-II178NCADA mice (30-50% 129/Sv coat color). (B) Arrows point to an outpouching of cells located near the tip of a jejunal villus. These cells lack a clearly defined apical brush...
looked like blisters. The border between "columns" of UEA-1-positive and UEA-1-negative enterocytes was not well defined in polyclonal villi (Fig. 4 C). Nonetheless, the heights of wholly UEA-1-positive villi were similar to the heights of wholly UEA-1-negative villi.

These changes were evident in B6>*129/Sv-I178NCADΔ mice produced from 5 of 8 independent ES cell lines (n = 3–5 mice analyzed/cell line; clone color chimerism = 20–50% for the progeny of the 5 cell lines compared to 40 to >95% for the 3 cell lines that yielded animals without a phenotype). The severity of the phenotypes observed in mice produced with each of the 5 cell lines varied along their duodenal-ileal axis. The most pronounced changes invariably occurred in the distal third of the small intestine (operationally defined as ileum). This regional variation parallels the known cephalocaudal pattern of I-FABP<sup>1178</sup> to <sup>129</sup> expression.

A Range of Phenotypes Can be Ascribed to a 129/Sv-I178NCADΔ Genotype. Sections were prepared from the entire duodenal-ileal axis of 6–8-wk-old B6>*129/Sv mice and comparably aged B6>*129/Sv-I178NCADΔ chimeras (n = 2–4 mice from all 5 cell lines). Hematoxylin and eosin staining revealed that the morphology of enterocytes in normal B6>*129/Sv mice did not change appreciably from the crypt-villus junction to the apical extrusion zone. These cells have a uniform size, contain a homogeneously stained cytoplasm, possess a distinct apical brush border, and are tightly adherent to their neighbors (Fig. 5 A). Less than one in 750 villi show evidence of active cell extrusion (as defined by Madara, 1990). When detected, cells are extruded singly or in pairs from the villus tip. In contrast, hematoxylin- and eosin-stained sections of B6>*129/Sv-I178NCADΔ small intestine revealed a variety of histologic abnormalities. The severity varied between the five cell lines but was identical for all mice derived from a given line. In the duodenum and proximal jejunum, cross-sections of some villi revealed bands of distended cells that lacked a distinctive brush border (Fig. 5 B). Some villi contained bands of enterocytes with normal morphology and adjacent bands of cells with large cytoplasmic vacuoles or enterocytes with a condensed cytoplasm and a pyknotic nucleus (Fig. 5 C). These latter features are consistent with entry into a death program. In the distal jejunum, villi often contained clusters of enterocytes that had lost their normal cell-cell contacts and appeared to be extruding into the lumen (Fig. 5 D). Villi in the proximal ileum contained groups of enterocytes that had lost their attachment to each other and to the underlying lamina propria (Fig. 5 E). Staining adjacent sections with UEA-1 allowed us to correlate all of these changes with a 129/Sv-I178NCADΔ genotype.

Some jejunal villi contained bands of UEA-1-positive cells that had separated from the basement membrane, creating a highly vacuolated space between enterocytes and the villus core (Fig. 5 F). This separation was initially expressed as an increase in clear subnuclear vesicles in the lower third of the villus, followed by a more pronounced separation at the midpoint of the villus. The lamina propria side of the basement membrane was lined with mesenchymal cells (Fig. 5 F). While cell-matrix interactions were perturbed, cell-cell contacts appeared to be intact. One possible explanation for this phenotype was that the epithelial sheet had separated from the mesenchyme due to basement membrane dissolution. A study with cultured cells had shown previously that suppression of cadherin function results in secretion of a matrix-degrading, urokinase-type, plasminogen activator (Frixen and Nagamine, 1993). Therefore, sections containing polyclonal villi with these abnormalities were incubated with antisera specific for laminin, collagen type IV, or heparin sulfate proteoglycan. There were no detectable differences in the distribution or levels of these extracellular matrix components under UEA-1-positive 129/Sv-I178NCADΔ-enterocytes and UEA-1-negative B6 enterocytes, indicating that the basement membrane was intact (data not shown).

Analysis of multiple UEA-1-positive ileal villi revealed that a range of phenotypes was often evident along the basilar-to-apical axis of a single villus (Fig. 5 G). Cells at the base of a villus were "piled-up" on each other. As cells migrated up the villus, vacuolated regions appeared between adjacent enterocytes and between enterocytes and the basement membrane (Fig. 5 G). Groups of enterocytes separated from the underlying lamina propria before they had reached the normal site of exfoliation at the apical extrusion zone (Fig. 5 G). These cells lost their differentiated phenotype as they approached the villus tip; i.e., they became spindle-shaped, brush border staining was weak to absent, and Golgi staining was disorganized. Some polyclonal ileal villi contained bands of UEA-1-positive cells that bore no resemblance to any of the known intestinal epithelial lineages. These cells were highly vacuolated and contained few discernable nuclei (Fig. 5, H and I).
Careful analysis of the duodenal-ileal axis of B6<sup>+</sup>129/Sv-II178NCADA mice failed to reveal any bands of B6 enterocytes with any of the histological abnormalities described above.

Loss of Cadherin-mediated Cell Adhesion Disturbs the Intestinal Epithelial Barrier. The integrity of the intestinal epithelial barrier is thought to be regulated by the highly organized apical junctional complex and its associated terminal web of microfilaments (cf. Weiser et al., 1986; Madara, 1990). Electron microscopic studies of normal intestinal epithelial cells undergoing cell division or extrusion indicate that their apical junctional complexes are rapidly remodeled (Madara, 1990; Jinguji and Ishikawa, 1992). Experiments in cultured cells suggest that E-cadherin is essential for this type of remodeling (Gumbiner et al., 1988; Watabe et al., 1994). Therefore, we anticipated that disrupting cadherin function in enterocytes would greatly compromise barrier functions. Analysis of 6–8-wk-old B6<sup>+</sup>129/Sv-II178NCADA mice (n = 3–5 animals/cell line; 5 cell lines surveyed) revealed evidence of such compromise. First, as noted above, cell–cell contacts were disturbed. Second, PAS and Alcian blue staining disclosed that the surface mucus layer overlying 129/Sv-II178NCADA but not B6 epithelium was disrupted (data not shown). This mucus layer protects the epithelium from mechanical, chemical, and microbiologic damage (Weiser et al., 1986). Third, clusters of Gram-negative bacteria were found infiltrating between poorly adherent villus-associated enterocytes (Fig. 6 B). Surveys of serial sections demonstrated that the bacteria were restricted to UEA-1-positive, 129/Sv-II178NCADA cells. Flushing the intestine before fixation did not remove the bacteria. They were not detected in nonchimeric littermates housed in the same microisolator cages or in normal, age-matched B6<sup>+</sup>129/Sv chimeras maintained in the same barrier facility. Their presence could reflect entry of bacteria and/or other luminal antigens through direct breaches in the epithelial barrier. They can not be ascribed to an immunologic response to the Xenopus NCADA itself since I-FABP<sup>+</sup> is activated on embryonic day 15, well before development of the immune repertoire in mice (e.g., for reviews see Goodnow, 1992; Lo, 1992).

Mechanisms

The Levels and Distribution of Endogenous Cadherins. To begin to explore the mechanisms responsible for producing these changes in differentiation and adhesion, sections were prepared from the small intestine of 6–8-wk-old B6<sup>+</sup>129/Sv-II178NCADA mice that had been generated with two independent ES cell lines (n = 3 mice/cell line; 30–50% 129/Sv). Fig. 2 D shows a polyclonal ileal villus incubated with the E-cadherin mAb and UEA-1. E-cadherin is concen-
Figure 7. NCADΔ expression is associated with disruption of the actin cytoskeleton and loss of polarity in villus-associated enterocytes. (A) Section prepared from the proximal ileum from a B6*129/Sv mouse and stained with a mouse β-actin mAb (visualized with Cy3-labeled sheep anti-mouse Ig). A prominent band of immunoreactive protein is associated with the terminal web and brush border of enterocytes located at the villus tip (closed arrows). Weak staining is detectable along the basolateral surfaces of these cells (e.g., open arrow). Lymphocytes located in the lamina propria (closed arrowheads) react with the sheep anti-mouse Ig. (B) Section of a proximal ileal villus from a B6*129/Sv-II178NCADΔ mouse. Apical brush border and terminal web staining is absent. There is pronounced increase in immunoreactive β-actin in the cytoplasm of these 129/Sv-II178NCADΔ enterocytes. (C) Section containing proximal ileal villi from the normal B6*129/Sv mouse shown in A. The section was incubated with peroxidase-conjugated Glycine max agglutinin (GMA; visualized with DAB; counterstain = hematoxylin). There is prominent staining of glycoconjugates located in the apical brush border and supranuclear Golgi apparatus. (D) Section of proximal ileum from the mouse in B. The section is stained with GMA as in C. As 129/Sv-II178NCADΔ enterocytes migrate up the villus, they lose their distinctive Golgi and brush border staining and acquire cytoplasmic vacuoles that do not react with the lectin. Bar, 25 μm.
trated at points of contact between normal B6 enterocytes. E-cadherin levels increase as these cells migrate toward the villus tip. The steady state level of E-cadherin is reduced dramatically in the adjacent band of 129/Sv-II178NCADA enterocytes. Abnormal, subnuclear clumps of E-cadherin immunoreactivity were observed in UEA-1-positive enterocytes, especially if the cells had separated from the underlying mesenchyme (Fig. 2 E).

Sections were also incubated with the pan-cadherin antiserum that reacts with the cytoplasmic domains of NCADA and endogenous wild-type cadherins. Despite the decrease in E-cadherin levels (Fig. 2 D), total cellular cadherin concentrations (NCADA plus endogenous cadherins) were increased in villus-associated 129/Sv-II178NCADA compared to B6 enterocytes (Fig. 2, C and F). This finding established that the II178NCADA transgene was actively expressed in 129/Sv enterocytes. In addition, the pan-cadherin antibody revealed large clumps of immunoreactive protein in 129/Sv-II178NCADA enterocytes at sites of cell–cell contact and throughout their cytoplasm (compare panels P and C in Fig. 2). Adjacent bands of B6 enterocytes, as well as epithelial cells in B6 and 129/Sv-II178NCADA crypts, had no detectable abnormalities in the level or intracellular distribution of cadherins (data not shown).

**Actin Distribution and Cell Polarity.** α-Catenin, β-catenin, and γ-catenin bind to the cytosolic domain of E-cadherin and link it to the cytoskeleton (Ozawa et al., 1989; Nagafuchi and Takeichi, 1988). Studies with cultured cells indicate that the interactions between cadherins, catenins, and the actin-based cytoskeleton are essential for cell adhesion and maintenance of cell polarity (Nagafuchi and Takeichi, 1988; Ozawa et al., 1990; Watabe et al., 1994). Therefore, sections of normal B6**--129/Sv and B6**--129/Sv-II178NCADA jejenum and ileum were incubated with a mouse β-actin mAb. The results indicate that the actin cytoskeleton becomes disorganized as contact is diminished between 129/Sv-II178NCADA enterocytes during their migration up the villus: β-actin levels are reduced and the protein’s distinctive localization at the terminal web is replaced by a diffuse cytoplasmic distribution (compare panels A and B in Fig. 7).

Actin may be particularly important for maintaining polarity in enterocytes, since it is the predominant component of microvilli and the terminal web (Drenchkahn and Dermietzel, 1988). Lectins are remarkably sensitive tools for defining variations in the differentiation program of each of the intestine’s four principal cell lineages and for identifying changes in protein compartmentalization (Falk et al., 1994, 1995a). The N-acetyl-D-galactosaminyl-specific lectin, *Glycine Max* agglutinin (GMA) recognizes glycoconjugates that accumulate in the apical brush border and Golgi apparatus of enterocytes (Falk et al., 1994). 129/Sv-II178NCADA enterocytes have markedly decreased or absent brush border staining with GMA. Moreover, Golgi staining is punctate and disorganized compared to B6 enterocytes (Fig. 7, C and D). Similar results were obtained with four other lectins that have distinct carbohydrate specificities—*Helix pomatia* agglutinin, *Arthrocarpus integrifolia* agglutinin, *Dolichos biflorus* agglutinin, and *Molluscella laevis* lectin. These findings are consistent with a defect in the polarized sorting of apical membrane proteins in NCADA-producing enterocytes.

NCADA-mediated perturbations of the cytoskeleton and polarized distribution of cellular proteins may have contributed to the high incidence of neonatal lethality observed in our pilot experiments. Limited analyses of moribund postnatal day 1 animals revealed numerous villi with bands of grossly disturbed cells having characteristics similar to those observed in the proximal ileum of adult low percent B6**--129/Sv-II178NCADA chimeras (Fig. 5, G–I). The absence of an organized enterocyte brush-border membrane is a feature of microvillus inclusion disease, an autosomal recessive disorder associated with high infant mortality (Cutz et al., 1989).

Together, these findings indicate that endogenous cadherins are needed to maintain an enterocyte’s polarity during its 48 h residence on the villus. The findings also lend support to the notion that differentiation of this lineage is not an autonomous process (Duluc et al., 1994) but rather an active process supported by a molecular cross talk which includes cadherin-dependent cell–cell and/or cell–matrix interactions.

**Cell Proliferation in Crypts.** Analysis of hematoxylin- and eosin-stained sections prepared from the most perturbed regions of B6**--129/Sv-II178NCADA intestine indicated that crypts were deeper and had increased numbers of M-phase cells (mean = 4 mitotic figures/longitudinal crypt section in B6**--129/Sv-II178NCADA ileum compared to 1 mitotic figure/ileal crypt in comparably aged B6**--129/Sv mice; n = 30 crypt sections counted per mouse; 2 mice/genotype) (Fig. 8, A and B). Increased proliferation in ES-derived crypts is noteworthy since I-FABP**--1178 to **128 is not active in their epithelial cell populations. It suggests that there is a signaling pathway which operates along the crypt-villus axis to sense any changes in the census of villus-associated enterocytes.

![Figure 8](image.png) Disrupting cadherin function results in increased proliferation in crypts, an increased rate of cell migration, and precocious induction of programmed cell death. (A) Hematoxylin- and eosin-stained section of ileal crypts from a 6-wk-old B6**--129/Sv chimeric mouse. (B) Section from a comparably aged B6**--129/Sv-II178NCADA chimeric-transgenic animal showing hyperproliferative ileal crypts. M-phase cells are more prevalent (closed arrows) and the crypts are deeper. The sections shown in A and B were prepared from the same position along the duodenal-ileal axis and photographed at the same magnification. (C) Section of a polyclonal jejunal villus from a 6-wk-old B6**--129/Sv-II178NCADA mouse injected with BrdU 60 h before sacrifice. The section was incubated with goat anti-BrdU (visualized as in C). The closed arrows point to BrdU-positive cells with nuclear DNA fragmentation suggesting programmed cell death. (Double staining with UEA-1 established that these cells had a 129/Sv-II178NCADA genotype, data not shown). (D) Double exposure of the same section as shown in C after staining with biotin-conjugated UEA-1 (visualized with FITC-conjugated extravidin). A comparison of C and D demonstrates that BrdU-positive 129/Sv-II178NCADA enterocytes (yellow-green plus red) are located higher up on the villus than BrdU-positive B6 enterocytes (red only). (E) Section of ileum from a 6-wk**--129/Sv-II178NCADA mouse stained with anti-BrdU sera (visualized as in C). The closed arrows point to BrdU-positive cells with nuclear DNA fragmentation suggesting programmed cell death. (Double staining with UEA-1 established that these cells had a 129/Sv-II178NCADA genotype, data not shown). (F) A TUNEL assay performed on a section of B6**--129/Sv-II178NCADA ileum (counterstained with crystal violet). Reconstruction of serial sections revealed that the band of TUNEL-positive (apoptotic) cells (dark brown, closed arrows) had a 129/Sv-II178NCADA genotype. Note that no TUNEL-positive cells are detectable in an adjacent band of B6 enterocytes located on the same polyclonal villus. Bar, 25 μm.
their state of differentiation, their rate of exfoliation, and/or their death programs, and to initiate compensatory proliferative responses. The nature of this pathway remains to be defined.

Migration Rates and Apoptosis. As noted above, analysis of intestinal whole mounts and tissue sections revealed that NCADA production does not affect villus height. An increase in crypt cell production without a concomitant change in villus height could reflect a number of mechanisms: (a) NCADA-producing enterocytes could migrate more rapidly along the crypt-villus axis and be extruded at an accelerated rate from the apex of the villus and/or (b) NCADA-positive enterocytes could be lost during their migration along the villus, either through extrusion at ectopic sites and/or by precocious entry into a death program.

To determine if NCADA disturbed migration rates, we first analyzed migration rates in 6-wk-old normal B6 mice and in B6<sup>++</sup>/Sv chimeras. Animals were given a single intraperitoneal injection of the thymidine analog 5'-bromo-2'-deoxyuridine (BrdU) to label crypt epithelial cells during S-phase. Mice were sacrificed 1.5, 6, 12, 24, 48, 60, 72, or 96 h later and sections of crypt-villus units in duodenal, jejunal, and ileal segments were incubated with an anti-BrdU sera to determine the distance that BrdU-labeled cells had migrated. In all regions of the duodenal-ileal axis, enterocytes located at the leading edge of the band of BrdU-positive cells reach the crypt-villus junction within 12 h while the lagging edge of the band passes through this junction within 48 h. In the ileum, the leading edge of BrdU-positive enterocytes reaches the villus tip by 60 h. At this time point, the band of BrdU-positive cells covers the upper two thirds of the villus. By 72 h, only the upper quarter of ileal villi contain labeled cells, indicating that a large number of enterocytes have been extruded from the villus tip. In the duodenum and jejunum where villi are longer, BrdU-labeled cells take 60 h to arrive at midportion of the villus, 72 h to reach the villus tip, and are rarely seen at 96 h (data not shown). These migration rates are not affected by genetic background:

- the number of labeled cells/crypt-villus unit/time point,
- and the distance they migrate/unit of time are the same for UEA-1-positive 129/Sv-enteroctyes and for UEA-1-negative B6 enterocytes located on the same polyclonal villus (data not shown).

When B6<sup>++</sup>/Sv-I1178NCADA mice were sacrificed 60 h after treatment with BrdU, it was apparent that the band of BrdU-labeled enterocytes in a 129/Sv-I1178NCADA stripe was significantly shorter than the band of BrdU-labeled cells in an adjacent B6 stripe. For example, BrdU-labeled UEA-1-positive cells were located in the upper quarter of an ileal villus, a position normally only reached after 72 h in normal 129/Sv ileal villi. In contrast, two thirds of the adjacent B6-derived stripe contained labeled cells (n = 4 comparably aged mice derived from two independent 129/Sv-I1178NCADA cell lines; at least 10 polyclonal villi surveyed/animal; e.g., see Fig. 8, C and D). These results indicate that 129/Sv-I1178NCADA enterocytes migrate at an accelerated rate.

A large number of BrdU-positive 129/Sv-I1178NCADA enterocytes in distal jejunal and ileal villi had fragmented appearing nuclei, suggesting entry into a cell death program (Fig. 8 E). TdT-mediated dUTP-nick end labeling (TUNEL) assays were performed to evaluate this finding further (Gavrieli et al., 1992; Hall et al., 1994). In normal 6-8-wk-old B6<sup>++</sup>/Sv mice, this assay only labels a few cells located at the extreme tips of villi plus a few scattered cells in the crypts (data not shown). In age-matched B6<sup>++</sup>/Sv-I1178NCADA mice, bands of UEA-1-positive enterocytes were often labeled in the TUNEL assay (Fig. 8 F). In some cases, these bands of TUNEL-positive cells extended along the entire length of the villus. This pattern of labeling was not evident in adjacent B6 enterocytes (Fig. 8 F). It is important to note that these increases in apoptosis were only observed in those areas of the duodenal-ileal axis where cell-cell adhesion was most severely disrupted (i.e., proximal ileum; n = 6 mice representing 3 independent ES cell lines). When cell-matrix interactions were disrupted without associated loss of cell-cell contacts (e.g., the jejunal villus shown in Fig. 5 F), bands of TUNEL-positive cells were never found. Together, these data indicate that loss of cadherin-mediated cell-cell contacts produces precocious induction of programmed cell death and suggests that cadherins can function as survival factors for enterocytes in vivo.

Discussion

We have expressed a dominant-negative N-cadherin mutant protein in postmitotic, villus-associated enterocytes. Our results provide an in vivo demonstration that cadherins can function to actively maintain the state of differentiation of an epithelial cell lineage, to control its adhesive properties and rate of migration, and to regulate its death program.

The Effect of NCADA on Enterocyte Adhesion and Migration. Cell adhesion has been disrupted by expressing a N-cadherin mutant lacking the extracellular domain in Xenopus embryos (Kinter, 1992; Dufour et al., 1994; Holt et al., 1994), in a mouse keratinocyte cell line (Fujimori and Takeichi, 1993), and now in villus-associated enterocytes. When expressed at high levels, Xenopus NCADA competes with and prevents &alpha-catnien binding to E-cadherin in Xenopus embryos (Kinter, 1992). In contrast, expression of a comparable chicken N-cadherin dominant-negative mutant in the mouse keratinocyte cell line does not appear to alter interactions between endogenous cadherins and catenins. Rather, immunocytochemical studies demonstrated that production of this NCADA mutant results in a pronounced reduction in the levels of endogenous mouse E-cadherin at sites of cell-cell contact (Fujimori and Takeichi, 1993). We noted a similar reduction in endogenous E-cadherin levels when NCADA expression was directed to villus-associated mouse enterocytes in vivo (Fig. 2, D and E). Fujimore and Takeichi (1993) hypothesized that (a) NCADA may replace endogenous cadherin-catenin complexes at adherens junctions and (b) the mutant protein may interfere with formation of large supramolecular E-cadherin arrays (cores) which develop after intact endogenous cadherins initially accumulate at sites of cell-cell contact through their intercellular homophilic interactions. They proposed that this interference could actively remove preexisting endogenous cadherins from these sites of cell-cell contact if the mutant cadherin has a higher affinity for cytoskeletal "receptors." The presence of complexes composed of a mutant cadherin that lacks an extracellular domain prevents cell adhesion. Our results support these notions. Studies with an E-cadherin mAb disclosed that cellular E-cadherin levels are reduced at the
basolateral surfaces of villus-associated 129/Sv-NCADA enterocytes. Moreover, when a pan-cadherin antibody was used to monitor the distribution of NCADA (plus endogenous cadherins), we noted (a) a pronounced increase in total cellular cadherin levels; (b) more immunoreactive protein at sites of cell–cell contact; and (c) an abnormal cytoplasmic distribution (Fig. 2 F).

Assembly of endogenous cadherin-catenin complexes appears to be a dynamic process. Large pools of cadherin-bound and cadherin-independent catenins are present in cultured cells (Hinck et al., 1994; Näthke et al., 1994). Several observations support the hypothesis that endogenous cadherins and cadherin-catenin complexes must turn over before a critical mass of NCADA can accumulate at specific sites within enterocytes to disrupt adhesion. I-FABP

Disrupting the Function of Mouse Cadherins In Vivo

Prevent Precocious Programmed Cell Death: Implications for the Pathogenesis of Intestinal Neoplasia. Death programs may be activated or suppressed by signals from the environment and prevention of programmed cell death may require continuous inhibition by locally acting "survival factors" (Raff, 1992). Loss of integrin-mediated cell–cell or cell–matrix contacts can lead to apoptosis in cultured cells (Meredith et al., 1993; Bates et al., 1994; Frisch and Francis, 1994; Montgomery et al., 1994; Re et al., 1994). This phenomenon has been termed "anoikis" and may be a normal mechanism which prevents cellular reattachment at inappropriate sites, thereby maintaining proper tissue organization and cellular census (Raff, 1992; Frisch and Francis, 1994; Ruoslahti and Reed, 1994).

Morphological as well as DNA fragmentation data indicate that programmed cell death is normally restricted to the apical extrusion zone located at the villus tip (Gavrelli et al., 1992; Hall et al., 1994). Analysis of B6×129/Sv-NCADA mice revealed that loss of cadherin-mediated cell–cell and/or cell–matrix interactions can trigger induction of programmed cell death along the entire villus. This suggests that entry into an apoptotic pathway is actively suppressed in cells distributed along the length of the villus and that cadherins represent one class of survival factors essential for active suppression of this death program. The nuclear organization of enterocytes distributed along the villus of normal B6×129/Sv mice suggests that preparation for death is a normal part of the lineage's terminal differentiation program: i.e., chromatin condensation and nuclear margination, early markers of apoptosis (Wyllie et al., 1980), accompanied migration-associated differentiation (Fig. 5 A).

Functional E-cadherin complexes contain either α- and β-catenin, or α-catenin and plakoglobin (Hinck et al., 1994). If these complexes are negative regulators of cell death in villus-associated enterocytes, then it is possible that the tumor suppressor APC could be a positive regulator of death. APC and E-cadherin compete for binding to the same internal, armadillo-like repeats of β-catenin (Hülsken et al., 1994; Rubinfeld et al., 1993; Su et al., 1993). Therefore, a dynamic equilibrium may exist between pools of free β-catenin, β-catenin:E-cadherin complexes, and β-catenin:APC complexes (Hinck et al., 1994; Hülsken et al., 1994). We hypothesize that a shift in the equilibrium towards β-catenin:E-cadherin complexes may promote cell survival while a shift in the equilibrium towards APC:β-catenin could promote cell death. One way the β-catenin:APC complex could initiate apoptosis is through an α-catenin–mediated effect on the cytoskeleton since it has been shown that disruption of microfilaments results in apoptosis in vitro (Kolber et al., 1990).

The distribution of APC in colonic crypts and the effects of its inactivation are consistent with a role in preparing for cell death during terminal differentiation. APC is restricted to the basolateral surface of (human) colonocytes. Levels increase as these cells complete their migration-associated differentiation (Smith et al., 1993). Inactivation of APC appears to be an early event in the multistep journey to human colorectal neoplasia (Fearon and Vogelstein, 1990) and produces multiple small intestinal and colonic adenomas in
the Min mouse (Moser et al., 1990, 1992; Su et al., 1992). Since death is essential for maintaining appropriate cellular census, loss of such control would, at the very least, lead to hyperplasia.

Loss of E-cadherin function alone appears insufficient to produce intestinal neoplasms. Extensive analysis of 6–8-wk-old B6*S129/Sv-H7N1178NCADA mice (n = 15 animals derived from 5 stably transfected ES cell lines) failed to reveal any evidence of adenomas or carcinoma. Loss of cadherin-mediated cell adhesion is a late, rather than early, event in oncogenesis and correlates with tumor cell dedifferentiation in vitro and in vivo (Vliegheck et al., 1991; Frixen et al., 1991; for a review see Takeichi, 1993). Loss of potential death inducers such as APC and/or p53 may be needed before inactivation of E-cadherin can produce intestinal neoplasia. This could be tested by generating chimeric-transgenic mice with ES cells that contain both I-FABP-H7s to +2s/NCADA and Apcmin and noting if intestinal adenomas form earlier in postnatal life, if their numbers increase, or if they show a more malignant phenotype than in mice which only contain Apcmin. Alternatively, the lack of intestinal neoplasms in B6*S129/Sv-H7N1178NCADA mice may be due to the inability of “initiated” NCADA-producing enterocytes to achieve functional anchorage during their accelerated migration along the villus. Previous studies have shown that the expression of several oncogenes in villus-associated enterocytes can lead to their reentry into the cell cycle and dedifferentiation but not to neoplasia (Kim et al., 1993, 1994). The site of initiation of tumorigenesis in the gut may have to be the functionally anchored stem cell or one of its immediate descendants (Kim et al., 1993). Anchorage may be possible to genetically engineer outside of the stem cell zone if overexpression of E-cadherin is shown to retard migration rate along the crypt-villus axis.

Prospectus. Further immunocytochemical studies of the polyclonal villi in these chimeric-transgenic mice should help clarify the effect of loss of cadherin function on proteins such as APC, catenins, various adhesion molecules (e.g., integrins, ZO-1), and tyrosine kinases located at adherens junctions for a review see Ranscht, 1994). The results of such analyses should provide a conceptual basis for generating additional types of chimeric-transgenic mice to test the functions of proteins postulated to act upstream, downstream, or in parallel with cadherins to regulate the continuum of cell birth to cell death which exists along the crypt-villus axis (e.g., β-catenin; cf McCrea et al., 1993; Peifer et al., 1993). Finally, the experiments described in this paper have examined the role of cadherins in a differentiated cell lineage. It will be important to use other well defined promoters (Cohn et al., 1992; Simon et al., 1993; Crossman et al., 1994) to direct expression of NCADA to crypts as well as to villus-associated epithelial cells and assess the effects on establishment and maintenance of crypt-villus units.

The authors are indebted to Dave O’Donnell for his expert technical assistance, members of our lab for their many stimulating discussions, and Rolf Kemler and Chris Kinter for generously supplying us with needed reagents.

This work was supported in part by grants from the National Institutes of Health (DK37960 and DK30292).

Received for publication 7 December 1994 and in revised form 1 February 1995.

The Journal of Cell Biology, Volume 129, 1995
Frixen, U. H., and Y. Nagamine. 1993. Stimulation of urokinase-type plasminogen activator expression by blocking of E-cadherin-dependent cell-cell adhesion. Cancer Res. 53:3618–3623.

Frixen, U. H., J. Behrens, M. Sach, G. Eberle, B. Voss, A. Wards, D. Löchler, and W. Brichmeier. 1991. E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. J. Cell Biol. 113:173–185.

Fujimori, T., and M. Takeichi. 1993. Disruption of epithelial cell-cell adhesion by exogenous expression of a mutant functional N-cadherin. Mol. Biol. Cell. 4:437–447.

Gavrieli, Y., Y. Sherman, and S. A. Ben-Sasson. 1992. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J. Cell Biol. 119:493–501.

Geiger, T., V. Volberg, D. Ginsberg, S. Bitzur, I. Sabanay, and R. O. Hynes. 1993. Fibrinogen, T., and M. Takeichi. 1993. Disruption of epithelial cell-cell adhesion during development. J. Cell Biol. 120:457–468.

Gumbiner, B., B. Stevenson, and A. Grimaldi. 1988. The role of the cell adhesion molecule uvomorulin in the formation and maintenance of the epithelial junctional complex. J. Cell Biol. 107:1575–1587.

Hall, P. A., P. J. Coates, B. Ansari, and D. Hopwood. 1994. Regulation of cell adhesion by the mammalian gastrointestinal tract: the importance of apoptosis. J. Cell Sci. 107:3569–3577.

Hermiston, M. L., C. B. Latham, J. I. Gordon, and K. A. Roth. 1992. Simultaneous localization of six antigens in single sections of transgenic mouse intestine using a combination of immunofluorescence and fluorescence microscopy. J. Histochem. Cytochem. 40:1283–1290.

Hermiston, M. L., R. P. Green, and J. I. Gordon. 1993. Chimeric transferrin: a powerful tool for studying how the proliferation and differentiation programs of different epithelial cell lineages are regulated. Proc. Natl. Acad. Sci. USA. 90:8860–8867.

Hermiston, M. L., T. C. Simon, M. W. Crossman, and J. I. Gordon. 1994. Model systems for studying cell fate specification and differentiation in the gut epithelium. In Physiology of the Gastrointestinal Tract. Third Edition. L. R. Johnson, editor. Raven Press, New York. pp. 521–569.

Hinck, L., I. S. Näthke, J. Papoff, and W. J. Nelson. 1994. Dynamics of cadherin/catenin complex formation: novel protein interactions and pathways of complex assembly. J. Cell Biol. 125:1327–1340.

Hodivala, K. J., and F. M. Watt. 1994. Evidence that cadherins play a role in terminal differentiation. J. Cell Biol. 124:589–600.

Holt, C. E., P. Lemaire, and J. B. Gurdon. 1994. Cadherin-mediated cell-cell interactions are necessary for the activation of MyoD in Xenopus mesoderm. Proc. Natl. Acad. Sci. USA. 91:10844–10848.

Hulsken, J., J. Behrens, and W. Birchmeier. 1994. Tumor-suppressor gene activities that regulate the cell adhesion molecule uvomorulin in the formation and maintenance of the epithelial junctional complex. J. Cell Biol. 120:457–468.

Itzkowitz, S. H., M. Yuan, C. K. Montgomery, T. Kjeldsen, H. K. Takahashi, J. Behrens, and W. Brichmeier. 1994. Tumor-suppressor gene minogen activator expression by blockage of E-cadherin-dependent cell-cell adhesion. Proc. Natl. Acad. Sci. USA. 91:8865–8866.

Lo, D. 1992. T cell tolerance. Curt. Opin. Immunol. 4:711–715.
al. 1993. The APC gene product in normal and tumor cells. Proc. Natl. Acad. Sci. USA. 90:2846-2850.
Su, L.-K., B. Kinzler, B. Vogelstein, A. C. Preisinger, A. R. Moser, C. Luongo, K. A. Gould, and W. P. Dove. 1992. Multiple interstitial neoplasia caused by a mutation in the murine homolog of the APC gene. Science (Wash. DC). 256:668-670.
Su, L.-K., B. Vogelstein, and K. W. Kinzler. 1993. Association of the APC tumor suppressor protein with catenins. Science (Wash. DC). 262:1734-1737.
Sweetser, D. A., E. H. Birkenmeier, P. C. Hoppe, D. W. McKeel, and J. I. Gordon. 1988. Mechanisms underlying generation of gradients in gene expression within the intestine: an analysis using transgenic mice containing fatty acid binding protein/human growth hormone fusion genes. Genes & Dev. 2:1318-1332.
Takeichi, M. 1988. The cadherins: cell-cell adhesion molecules controlling animal morphogenesis. Development. 102:639-655.
Takeichi, M. 1991. Cadherin cell adhesion receptors as a morphogenetic regulator. Science (Wash. DC). 251:1451-1455.
Takeichi, M. 1993. Cadherins in cancer: implications for invasion and metastasis. Curr. Opin. Cell Biol. 5:806-811.
Tytulewicz, V. L. J., C. E. Crawford, P. K. Jackson, R. T. Bronson, and R. C. Mulligan. 1991. Neonatal lethality and lymphopenia in mice with a homozygous disruption of the c-abl protooncogene. Cell. 65:1153-1163.
Vestweber, D., and R. Kemler. 1985. Identification of a putative cell adhesion domain of uvomorulin. EMBO (Eur. Mol. Biol. Organ.) J. 4:3393-3398.
Vleminckx, K., L. Vakaet, Jr., M. Mareel, W. Fiers, and F. Van Roy. 1991. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. Cell. 66:107-119.
Walker, R. I., I. Brook, J. W. Costerton, T. MacVittie, and M. L. Myhal. 1985. Possible association of mucous blanket integrity with postirradiation colonization resistance. Radiat. Res. 104:346-357.
Watsabe, M., A. Nagafuchi, S. Tsukita, and M. Takeichi. 1994. Induction of polarized cell-cell association and retardation of growth by activation of the E-cadherin-catenin adhesion system in a dispersed carcinoma line. J. Cell Biol. 127:247-256.
Weiser, M. M., J. R. F. Walters, and J. R. Wilson. 1986. Intestinal cell membranes. Int. Rev. Cytol. 101:1-57.
Wheelock, M. J., and P. J. Jensen. 1992. Regulation of keratinocyte intercellular junction organization and epidermal morphogenesis by E-cadherin. J. Cell Biol. 117:415-425.
Wright, N. A., and M. Irwin. 1982. The kinetics of villus cells populations in the mouse small intestine: normal villus—the steady state requirement. Cell Tissue Kinet. 15:595-609.
Wyllie, A. H., J. F. R. Kerr, and A. R. Currie. 1980. Cell death: the significance of apoptosis. Int. Rev. Cytol. 68:251-305.
Zhou, L., C. R. Dey, S. E. Wert, M. D. DuVall, R. A. Frizzell, and J. A. Whitsett. 1994. Correction of lethal intestinal defect in a mouse model of cystic fibrosis by human CFTR. Science (Wash. DC). 266:1705-1708.