Cell-Cell Contacts Prevent Anoikis in Primary Human Colonic Epithelial Cells

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Background & Aims: **Colonic epithelial cells (CECs) receive important survival signals from the extracellular matrix and undergo detachment-induced apoptosis (anoikis) as soon as they lose their cell-matrix anchorage. In contrast to the established role of cell-matrix contact, the role of cell-cell contacts as a physiologic survival factor for CECs is less clear.** ***Methods:*** **Intact CEC crypts gently centrifuged to form a cell aggregate in which cell-cell contacts were maintained. Induction of apoptosis was assessed by Western Blot analysis, colorimetric assays, DNA electrophoresis, 4′,6-diamidino-2-phenylindole staining, and flow cytometry. Activation of survival pathways was analyzed by Western blot. The role of mitogen-activated protein kinase/extracellular signal–regulated kinase (MEK)/extracellular signal–regulated kinase (Erk)1/2, epidermal growth factor receptor, phosphatidylinositol 3-kinase (PI3-K), and Src signaling was investigated using specific inhibitors.** ***Results:*** **Despite a complete loss of cell-matrix adhesion after CEC isolation, activation of caspases was blocked and anoikis was prevented when cell-cell contacts were preserved. CECs with preserved cell-cell contacts exhibited a rapid dephosphorylation of focal adhesion kinase. Aggregated CECs had stable levels of active β-catenin and phosphorylated Akt, Erk1/2, and epidermal growth factor receptor, but CECs undergoing anoikis rapidly degraded β-catenin and dephosphorylated Akt. Inhibition of Src- and PI3-K–dependent signaling reversed the antiapoptotic effect of cell-cell contact preservation, while inhibition of the MEK pathway had no effect.** ***Conclusions:*** **Integrity of cell-cell contacts compensates for the loss of cell-matrix contact-mediated survival signals in CECs and prevents apoptosis. Cell-cell contact-triggered CEC survival involves antiapoptotic signaling through β-catenin-, Src-, and PI3-K/Akt- but not through MEK- and focal adhesion kinase–dependent pathways.**

Intestinal epithelial cells (IECs) constitute the largest surface of the human body and are of pivotal importance for the digestion and absorption of nutrients. They play a critical role in forming the primary barrier against microorganisms and toxins present in the intestinal lumen. Colonic epithelial cells (CECs) are generated from stem cells at the base of the crypt and migrate on the underlying basement membrane toward the intestinal lumen in 3–5 days, where apoptosis is initiated and cells finally lose anchorage and are shed into the lumen.[1–4]

Most nontransformed epithelial cell types undergo apoptosis when they lose their contact with the extracellular matrix (ECM),[5] a phenomenon termed "anoikis."[6] This special form of cell death is likely to be one of the mechanisms terminating the physiologic life cycle of IECs, because they gradually lose cell anchorage on their march toward the intestinal lumen.[7–12]

The central component of the apoptotic cell death machinery is a proteolytic cascade mediated by caspases.[13–17] During CEC anoikis, caspase-2 and caspase-9 are involved in the initiation of apoptosis and activate downstream effector caspase-7, caspase-3, and caspase-6.[18] Caspase activation results in the cleavage of various structurally and functionally essential intracellular substrate proteins[19] and culminates in characteristic morphologic changes becoming apparent in apoptotic cells.

Maintenance of structural and functional integrity of the intestinal epithelium requires highly dynamic cell-cell and cell-matrix interactions involving different types of surface receptors. Among these receptors, adhesion molecules, cadherins, and integrins play a major role by recognizing and interacting with other cell adhesion receptors on neighboring cells and by binding components of the ECM.[20–22] Besides providing mechanical anchorage to the cell, these structures also are of functional importance; they transduce signals from the ECM and neighboring cells that are critical for survival and proliferation. Loss of these signals frequently initiates apoptosis.

Abbreviations used in this paper: CEC, colonic epithelial cell; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; Erk, extracellular signal–regulated kinase; FAK, focal adhesion kinase; IEC, intestinal epithelial cell; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase/extracellular signal–regulated kinase; P-CEC, colonic epithelial cell cultured in pellets; PI3-K, phosphatidylinositol 3-kinase; S-CEC, colonic epithelial cell cultured in suspension.

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Most studies examining adhesion molecule signaling have focused on the mechanisms of cell-matrix interactions and their requirements for survival.[23,24] Recently, we established an isolation method allowing a quick purification of nonapoptotic colonic crypts.[25,26] We showed that rapid reestablishment of cell-matrix contacts protects isolated CECs from anoikis.[26,27] This advance allows us to study the importance of cell-cell contacts in regulating anoikis, a topic that has received limited attention in comparison with the studies on cell-matrix interaction.

Adjacent epithelial cells form tight junctions, which are critical for the maintenance of epithelial barrier and polarity function, and adherens junctions, structures predominantly composed of E-cadherin molecules that are linked to the actin cytoskeleton through an intracellular association with catenins.[28,29] Disruption of cadherin function in chimeric mice disrupts cell-cell adhesion and leads to an increased prevalence of apoptosis in CECs.[30,31] After loss of their cell-matrix anchorage, squamous epithelial cells and proximal tubular cells can be protected from anoikis by maintaining their cell-cell contacts. An additional blockade of E-cadherin binding by ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid, a hepatitis A virus–containing peptide,[32] or an anti–E-cadherin antibody[33,34] induced anoikis.

The pathways that cells use to communicate survival signals to each other are incompletely understood. There is a link between the E-cadherin system and the phosphatidylinositol 3-kinase (PI3-K) signaling pathway.[35] PI3-K and the serine-threonine kinase Akt are central elements[35] of cell-survival signaling, and a variety of extracellular stimuli lead to their activation.[36–39] Activation of Akt results in the downstream inactivation of a series of proapoptotic proteins.[40] Akt becomes activated upon the formation of intercellular contacts,[35,41] and preservation of cell-cell adhesion between mouse proximal tubular cells inhibits apoptosis in a PI3-K–dependent manner.[32] Recently it has been shown that the nonreceptor tyrosine kinase Src is a key intermediary kinase in the E-cadherin/PI3-K signaling pathway because its activity is required for the recruitment of PI3-K to sites of E-cadherin adhesion.[42]

So far, the role of cell-cell interactions in the control of human CEC survival was unclear. Therefore, we assessed whether cell-cell adhesions modulate the anoikis process of primary human CECs and elucidated the signaling pathways involved in cell-cell contact that triggered antiapoptotic signaling.

Materials and Methods

Isolation of CECs, Induction of CEC Apoptosis, and Generation of Pellets

CECs were isolated as previously described.[26] Briefly, normal human colonic mucosa from surgical specimens obtained from patients undergoing surgery for large bowel neoplasia (>10 cm from the tumor) were cut into small strips. Mucus was removed by incubation for 30 minutes at room temperature in 1 mmol/L dithiothreitol (Sigma, Taufkirchen, Germany) in 50 mL Hank's balanced salt solution (PAA, Linz, Austria). Mucosal strips were incubated in 1 mmol/L EDTA (Sigma) for 10 minutes at 37°C, briefly rinsed in Hank's balanced salt solution, and transferred to tubes containing fresh Hank's balanced salt solution at room temperature. Tubes were shaken vigorously 5–10 times. Mucosal strips were removed by passing the slurry over a coarse mesh (400 μm; Carl Roth GmbH, Karlsruhe, Germany). The suspension containing the detached CEC crypts was passed over the mesh filter (80-μm pore size; Sefar, Kansas City, MO), and intact CEC crypts were eluted by inverting the filter in serum-free culture medium (keratinocyte serum-free medium; Gibco-BRL, Eggenstein, Germany). Using this method, CECs were purified as intact crypts; it is important to note that cell-cell contacts within the CEC crypts were preserved, whereas cell-matrix contact was lost.

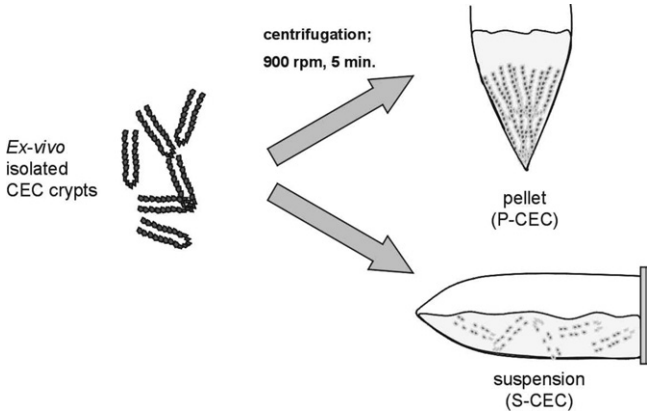

Figure 1. Experimental approach to investigate the role of cell-cell contacts in CEC anoikis. Isolated CECs were gently spun to form a cell aggregate (pellet), preserving CEC cell-cell contacts. Loss of cell-cell contacts was induced by keeping the cells in suspension.

To determine the role of cell-cell anchorage, freshly isolated CEC crypts were gently spun to form a cell aggregate (pellet) before disruption of CEC cell-cell contacts (Figure 1). Each pellet was generated from 3- to 5-mL aliquots of the suspension by centrifugation at 100*g* and 4°C for 5 minutes, in a manner similar to the method of Maldonado et al.[43] As control, CECs were liberated from isolated crypts by suspension in a polypropylene tube on a whip-shaker, inducing apoptosis as described previously.[44] Pellets and CECs in suspension were incubated at 37°C. At the indicated time points, cells were harvested by centrifugation at 4°C and analyzed further.

Transmission Electron Microscopy

Cells were fixed in 0.1 mol/L cacodylate-buffered Karnovsky solution (2.5% glutaraldehyde and 1% paraformaldehyde) overnight at room temperature, postfixed in 1% osmium tetroxide (2 hours) at pH 7.3, dehydrated in graded ethanols, and embedded in the EmBed-812 epoxy