ADAM-15 inhibits wound healing in human intestinal epithelial cell monolayers

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ADAM-15 inhibits wound healing in human intestinal epithelial cell monolayers. Am J Physiol Gastrointest Liver Physiol 288: G346–G353, 2005. First published September 9, 2004; doi:10.1152/ajpgi.00262.2004.—The disintegrin metalloproteases (or ADAMs) are membrane-anchored glycoproteins that have been implicated in cell-cell or cell-matrix interactions and in proteolysis of molecules on the cell surface. The expression and/or the pathophysiological implications of ADAMs are not known in intestinal epithelial cells. Therefore, our aim was to investigate the expression and the role of ADAMs in intestinal epithelial cells. Expression of ADAMs was assessed by RT-PCR, Western blot analysis, and immunofluorescence experiments. Wound-healing experiments were performed by using the electric cell substrate impedance sensing technology. Our results showed that ADAMs-10, -12, and -15 mRNA are expressed in the colonic human cell lines Caco2-BBE and HT29-Cl.19A. An ADAM-15 complementary DNA cloned from Caco2-BBE poly(A)+ RNA, and encompassing the entire coding region, was found to be shorter and to present a different region encoding the cytoplasmic tail compared with ADAM-15 sequence deposited in the database. In Caco2-BBE cells and colonic epithelial cells, ADAM-15 protein was found in the apical, basolateral, and intracellular compartments. We also showed that the overexpression of ADAM-15 reduced cell migration in a wound-healing assay in Caco2-BBE monolayers. Our data show that 1) ADAM-15 is expressed in human intestinal epithelia, 2) a new variant of ADAM-15 is expressed in a human intestinal epithelial cell line, and 3) ADAM-15 is involved in intestinal epithelial cell wound-healing processes. Together, these results suggest that ADAM-15 may have important pathophysiological roles in intestinal cells.

A disintegrin and metalloprotease (ADAM), also called metallopeptase/disintegrin/Cys-rich (MDC), is a family of membrane-anchored glycoproteins that present structural similarities with the snake venom metalloproteases (36, 37). Thirty-four ADAMs have been described in several species, including 19 in human. ADAM proteins consist of a prodomain, a metallopeptase domain, a disintegrin domain, a cysteine-rich region, an epidermal growth factor-like domain, a transmembrane domain, and a cytoplasmic tail (5, 36, 37). Proteins of this family have been shown to be involved in the processing of pro-tumor necrosis factor-α, L-selectin, a tumor necrosis factor receptor, transforming growth factor-α (28), and ADAM-10/Kuzbanian control cell determination and axon guidance in the nervous system via signaling through the Notch pathway (26, 30) and through shedding of ephrins (15). The cytoplasmic tail of ADAM proteins contains tyrosine phosphorylation sites and proline-rich domains that allow interactions with intracellular proteins involved in the cytoskeleton or signal transduction (19, 29, 31), suggesting that ADAMs could mediate intracellular signaling.

Among all the ADAM proteins that have been discovered, human ADAM-15 is the only one that has an arginine-glycine-aspartic acid (RGD) integrin-binding motif (19), suggesting a role of ADAM-15 in integrin binding and therefore in cell-cell interactions. It has indeed been shown that the disintegrin domain of ADAM-15 interacts with α5β1- and α3β1-integrins in an RGD-dependent manner (25, 40) and with α6β1-integrin independently of the RGD motif (9). In addition, overexpression of ADAM-15 in the fibroblastic cell line NIH3T3 has been found to enhance cell-cell interactions (16). ADAM-15 has also been shown to be involved in glomerular mesangial cell migration (22).

ADAM proteins have not been studied in intestinal epithelium. ADAM-15 expression in the intestine is only poorly documented (19), and nothing is known about its function in the intestine. Therefore, our aim was to examine ADAM proteins and especially ADAM-15 expression and its potential roles in human epithelial intestinal cells.

MATERIALS AND METHODS

Cell lines and cell culture. The Caco2-BBE cell line (7, 20, 24, 33) that differentiates at confluency into enterocyte-like cells was generously provided by Dr. M. S. Mooseker and the HT29-Cl.19A cell line (3, 23), which differentiates at confluency into ion-transporting colonic epithelial cells, was a generous gift from Dr. C. L. Laboisse. Caco2-BBE or HT29-Cl.19A cells were grown in high-glucose DMEM (Invitrogen, Grand Island, NY) supplemented with 14 mM NaHCO3, 10% (vol/vol) heat-inactivated fetal bovine serum (Invitrogen), and 1.5 μg/ml plasmocin (Invivogen, San Diego, CA). Transfected cells were maintained in the same medium containing 1.2 mg/ml G418 (Life Technologies, Rockville, MD). Cells were kept at 37°C, in 5% CO2, and 90% humidity. For biotinylation and confocal experiments, confluent cells in 75 cm2 were trypsinized, plated on porous filters (6-well or 12-well Transwell Clear, 0.4 μm porosity; Costar, VWR, Suwanee, GA). Caco2-BBE cells were then cultured...
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for at least 10 days, and HT-29.C1.19A cells were cultured for 20 days. For protein, membrane, or RNA extractions, Caco2-BBE cells were plated on six-well plates (Costar) and experiments were performed at postconfluency.

For experiments using the electric cell substrate impedance sensing (ECIS; Applied BioPhysics, Troy, NY) system, ECIS 8W1E arrays were used (Applied BioPhysics). Caco-2BBE cells were plated at 104 cells per well and experiments were performed 3 days after seeding when cells are at confluency.

RT-PCR for ADAMs-10, -12, and -15 expression. Expression of ADAMs-10, -12, and -15 in HT29.C1.19A and Caco2-BBE cells was determined by using a RT-PCR method with oligonucleotide primers specific for each of these ADAMs: ADAM-10, sense: 5'-GCCATATATATTTCCCTTGGCAGAC-3', anti-sense: 5'-CACGAGTAGGCGTTGACACTTGTGAGG-3', ADAM-12, sense: 5'-AGTTCAGAGAGCAGTTGAGAAA-3', anti-sense: 5'-TTGCGTTGTGCAATAGGCT-3', and ADAM-15, sense: 5'-GCTTCAGGTGTTCTCCTGACGAGGGA-3', anti-sense: 5'-GAGGTAGA-3'. Plasmids were then used (Applied BioPhysics). Caco-2BBE cells were plated at 106 cells per well and experiments were performed 3 days after transfection. Subconfluent Caco2-BBE cells were transfected by using Trojene Transfection reagent (Avanti, Alabaster, AL) according to the manufacturer’s instructions for 3–4 h in Opti-MEM I medium (Invitrogen). Transfectants were selected in medium with 1.2 mg/ml G418 (Life Technologies).

RT-PCR for ADAM-15 cytosolic domain. After RT of Caco2-BBE cells PolyA+ RNA, as described above, PCR were performed with primers allowing the elongation of the cytosolic domain of ADAM-15, sense: 5'-CTGCCACCTCAGCTAAG-3', anti-sense: 5'-CCCGGAGGTCAGAGGTGAAG-3'. After an initial denaturation step at 94°C for 5 min, PCR was carried out for 35 cycles under the following conditions: denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 10 min. PCR products were electrophoresed on ethidium bromide-stained 2% agarose gels in TAE.

Western blot analysis. For total protein extraction, cells were lysed for 30 min at 4°C in RIPA buffer [150 mM NaCl, 0.5% sodium deoxycholate, 50 mM Tris-HCl (pH 8.0), 0.1% SDS, 0.1% Nonidet P-40] supplemented with protease inhibitors (Roche Diagnostics, Mannheim, Germany). The homogenates were centrifuged at 13,000 g for 30 min at 4°C, and the supernatants were collected for Western blot analysis. Protein concentrations were determined by using the Folin assay (DC protein assay kit, Bio-Rad, Hercules, CA). Protein extracts were mixed in tricine sample buffer (Bio-Rad), boiled for 5 min, run on a 7.5% (Bio-Rad) or an 8% (WVR) polyacrylamide gel and then transferred to nitrocellulose membranes. Membranes were blocked overnight at 4°C or for 1 h at room temperature with 5% nonfat milk in blocking buffer and then incubated 1 h at room temperature with a rabbit polyclonal raised against the cytoplasmic tail of human ADAM-15 (1:1,000; R&D Systems, Minneapolis, MN). After washing, membranes were further incubated 1 h at room temperature with an anti-rabbit horseradish peroxidase-conjugated antibody (1:1,000; Amersham Biosciences, Piscataway, NJ). Membranes were washed again and immunoreactive proteins were detected on films using an enhanced chemiluminescence substrate according to the manufacturer’s instructions (Amersham Biosciences).

Cell surface biointinylation. Cells grown on filters were rinsed twice with PBS supplemented with 0.1 mM CaCl2 and 1 mM MgCl2. Apical or basolateral sides of the monolayers were incubated with freshly prepared sulfoconjugated biotin (EZ-link sulfo-NHS-biotin; Pierce, Rockford, IL) in PBS supplemented with 0.1 mM CaCl2 and 1 mM MgCl2 (1 mg/ml) for 30 min at 4°C. The reaction was quenched with 50 mM NH4Cl (5 min at room temperature). Cells were then scrapped and lysed for 30 min at 4°C in lysis buffer (immunoprecipitation kit, protein G; Roche Diagnostics) supplemented with protease inhibitors (Roche Diagnostics). After 30-min centrifugation (13,000 g, 4°C), protein concentrations were determined by using the Bio-Rad protein assay, and the supernatants were incubated with immobilized neutravidin (Pierce) overnight at 4°C to bind biotinylated proteins. After centrifugation, the beads were washed twice in PBS (20 min at 4°C), once in a buffer of 500 mM NaCl, 20 mM Tris pH 8.0, 0.5% Triton X-100, and 0.2% BSA and rinsed in PBS. The beads were then boiled 5 min in tricine sample buffer (Bio-Rad), and Western blot analysis was performed.

Membrane preparations. For membrane preparations, cells were washed twice in PBS and scrapped in PBS. After centrifugation (400 g for 5 min), the cell pellet was resuspended and carefully homogenized, with a douncer, in HEPES (5 mM) containing protease inhibitors, incubated for 30 min at 4°C, and then centrifuged at 13,000 g at 4°C for 30 min. The resulting pellet was suspended in PBS by repeated passage through an 18-gauge needle. Protein concentration in the membrane suspension and in total extracts was quantified by using the Bio-Rad protein assay.

Confocal immunofluorescence. Caco2-BBE cells grown on filters were washed twice with PBS pH 7.4 (Invitrogen) supplemented with 0.1 mM CaCl2 and 1 mM MgCl2 (PBS-Ca/Mg), and fixed in 4% paraformaldehyde (Electron Microscopy Sciences, Washington, PA) in PBS-Ca/Mg for 15 min at room temperature. After three washes in PBS-Ca/Mg, cells were reincubated at room temperature for 30 min in immunostaining buffer (PBS-Ca/Mg/BSA 3%/Triton X-100 0.1%) and incubated for 40 min at room temperature with Alexa Fluor 568-conjugated phalloidin (1 unit per filter; Molecular Probes, Eugene, OR) diluted in immunostaining buffer. Cells were then washed
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and exposed to a monoclonal mouse anti-ADAM-15 antibody directed to its ectodomain (1:20; R&D Systems), in immunostaining buffer, for 1 h at room temperature. After washing, cells were incubated with a goat anti-mouse antibody conjugated to Alexa Fluor 488 (1:200, Molecular Probes) for 1 h at room temperature. After washing, coverslips were mounted by using the Slowfade medium (Molecular Probes). Microscopy was performed by using a Zeiss epifluorescence microscope equipped with a Bio-Rad MRC600 confocal unit, computer, and laser scanning microscope image analysis software (Carl Zeiss, Jena, Germany).

Wound-healing assays. Wound-healing assays were performed with the ECIS (Applied BioPhysics) technology (12, 13, 18). The ECIS device is based on AC impedance measurements using weak and noninvasive AC signals as previously described in more detail (35). Attachment and spreading of cells on the electrode surface change the impedance in such a way that morphological information of the attached cells can be inferred. The measurement system consists of an eight-well culture dish (ECIS 8W1E plate), with the surface treated for cell culture. Each well contains a small active electrode (area = 5 × 10⁻⁴ cm²) and a large counter electrode (area = 0.15 cm²) deposited on the bottom of each well. A lock-in amplifier with an internal oscillator relays to switch among the different wells, and a personal computer controls the measurement and stores the data. The entire system was obtained from Applied Biophysics.

First, we determined the ideal frequency used to measure resistance of confluent Caco2-BBE monolayers. To do so, we performed a resistance measurement during a frequency scan on cells plated on ECIS 8W1E plates (Applied BioPhysics). Resistance measurement was the performed during a frequency scan on a naked electrode after cell trypsinization. The ideal frequency was determined by using the ratio of the log of resistances with cells over the log of resistances without cells. The ideal frequency is the frequency for which the ratio reaches a peak. For resistance measurements performed on Caco2-BBE cells, the frequency used was 500 Hz and the voltage was 1 V.

For the wound-healing assays, confluent Caco2-BBE monolayers and confluent Caco2-BBE that overexpressed ADAM-15 cultured on ECIS 8W1E plates were submitted to an elevated voltage pulse of 40 kHz, 4.5-V amplitude, and 30-s duration, which led to death and detachment of cells present on the small active electrode resulting in a wound normally healed by cells surrounding the small active electrode that have not been submitted to the elevated voltage pulse. Wound healing was then assessed by continuous resistance measurements for ~20 h. At the end of the experiments, pictures of the small active electrodes were taken to visualize cell repair and migration.

Flow cytometry. Flow cytometry experiments were performed to assess ADAM-15 overexpression in transfected Caco2-BBE cells. Twenty-four hours after transfection, Caco2-BBE cells were detached with 0.25% trypsin (Invitrogen) diluted 1:10 in PBS (without Ca²⁺ and Mg²⁺), pelleted by centrifugation, and resuspended in PBS containing 0.5% BSA (PBS/BSA). Cells (5 × 10⁵) were incubated with mouse monoclonal anti-ADAM-15 (ectodomain) antibody (1:20) in 100 µl PBS/BSA for 1 h at 4°C, washed twice with PBS/BSA, and stained with a FITC-conjugated goat anti-mouse IgG (1:100; Santa Cruz Biotechnology, Santa Cruz, CA) in 100 µl PBS/BSA for 1 h at 4°C. Nonspecific fluorescence was determined with FITC-conjugated goat anti-mouse IgG. After two washes in PBS, fluorescence was analyzed on a FACScan cytofluorometer (Becton Dickinson) with Cell Quest software. Seven thousand cells gated on a FSC/SSC dot plot were assayed.

RESULTS

ADAMs-10, -12, and -15 mRNAs are expressed in the epithelial intestinal cell lines Caco2-BBE and HT29-CI.19A. To assess the expression of ADAMs in epithelial intestinal cells, we performed RT-PCR experiments on epithelial intestinal cell lines, as described in MATERIALS AND METHODS. As shown in Fig. 1, ADAMs-10, -12, and -15 mRNA are expressed in the human colonic cell line Caco2-BBE. As expected, the size of ADAMs-10, -12 and -15 PCR products were found to be ~490, 350, and 500 base pairs (bp), respectively. The same results were found in HT29-CI.19A cells (not shown).

Caco2-BBE cells express two ADAM-15 isoforms. Among all ADAMs described, human ADAM-15 has the characteristic to possess an RGD integrin binding motif in its disintegrin domain (19). We therefore focused on ADAM-15, and the full-length cDNA encoding ADAM-15 in Caco2-BBE cells was cloned and sequenced as described in MATERIALS AND METHODS. Our results showed that the cloned ADAM-15 cDNA presents a 70-bp deletion in the region encoding the cytoplasmic tail (from 2,208 to 2,277 bp), in ADAM-15 cDNA (GenBank accession no. AY518542) compared with published ADAM-15 cDNA (GenBank accession nos. 2208386A, AAC50404, AAM44189, AAP8766, Q13444, NP_003806, and BC014566) (Fig. 2A). This 70-bp deletion results in a change in the open reading frame of ADAM-15 mRNA, leading to the generation of a stop codon (UAG) at 2,317 bp instead of the stop codon UGA, located at 2,443 bp (Fig. 2A). As represented in Fig. 2B, the ADAM-15 protein generated from this mRNA is therefore shorter (772 amino acids instead of 814), and its cytoplasmic tail differs after amino acid 735. To further investigate the expression of this mRNA in Caco2-BBE cells, we performed RT-PCR experiments using primers allowing the elongation of the cytoplasmic tail of ADAM-15, as described in the MATERIALS AND METHODS. Two PCR products were found: one corresponding to the full-length cytoplasmic tail (406 bp, long isoform) and one corresponding to the 70-bp deletion-containing cytoplasmic tail (336 bp, short isoform) (Fig. 2C). Figure 2D represents the amino acids sequence of ADAM-15 cytoplasmic tail for the long (upper sequence) and for the short (lower sequence) isoforms. The cytoplasmic tail of ADAM-15 presents some motifs suggesting that ADAM-15 might transduce intracellular signals. Potential proline-rich domains (19, 31) are represented in green (Fig. 2D). Tyrosines represented in blue (Y715 and Y735) have been described as phosphorylated (29) and serine and threonine represented in red are potential phosphorylation sites according to the NetPhos 2.0 Server (Fig. 2D). The cytoplasmic tail of the short isoform of ADAM-15 is 42 amino acids shorter; it comprises 61 amino acids, whereas the cytoplasmic tail of the long

Fig. 1. A disintegrin and metalloproteases (ADAMs)-10, -12, and -15 mRNA are expressed in human intestinal cell lines. Samples isolated from the human epithelial colonic HT29-CI.19A cell line were subjected to RT-PCR using GAPDH, ADAM-15-, ADAM-12-, and ADAM-10-specific primers as described in MATERIALS AND METHODS. The RT-PCR products were analyzed by agarose gel electrophoresis. Numbers on the right are the molecular size of standards in base pairs.
isoform comprises 103 amino acids (Fig. 2D). This shorter cytoplasmic tail possesses tyrosines 715 and 735, and, according to the NetPhos 2.0 Server, it contains two serines and one threonine that are potential phosphorylation sites. However, this short cytoplasmic tail lacks all the predicted proline-rich domains found in the long cytoplasmic tail, and the environment of tyrosine 735 is not the same (Fig. 2D), suggesting that this tyrosine might not be phosphorylated in the short isoform. This suggests that the short and long isoforms of ADAM-15 might transduce different intracellular signals.

ADAM-15 protein is expressed in human intestinal epithelial cells. ADAM-15 expression in the intestine was further examined at the protein level. Immunoblot experiments performed on human biopsy whole cell extracts showed that both pro form (110 kDa) and mature form (85 kDa) of ADAM-15 protein are expressed in human small intestine (Fig. 3A). Surprisingly, only one band of ~90 kDa was revealed in human colon extracts (Fig. 3A). The enterocyte-like Caco2-BBE cell line was then used as a model system to assess ADAM-15 expression in human intestinal epithelial cells. Immunoblot experiments performed on whole cell extracts as well as on membrane preparations from Caco2-BBE cells showed that both pro form and mature form of ADAM-15 are present in the intracellular compartment as well as at the cell surface membrane (Fig. 3B).

To assess the localization of ADAM-15 in Caco2-BBE cells, we performed immunofluorescence staining using a mouse anti-ADAM-15 antibody raised against the ectodomain. As shown in Fig. 4A, ADAM-15 was found to be expressed on the cell membrane as well as in the intracellular compartment. Within the membrane, ADAM-15 was found to be expressed in both apical and basolateral compartments, although the staining was more intense in the apical compartment (Fig. 4A).
These data were confirmed by cell surface biotinylation experiments, which showed that although both forms of ADAM-15 are expressed in both apical and basolateral compartments, the mature form of ADAM-15 is expressed in a much higher level in the apical compartment (Fig. 4B).

**ADAM-15 regulates intestinal epithelial cell wound-healing.** ADAM-15 has been described as having a protease activity resulting in type IV collagen and gelatin degradation (22) and in CD23 shedding (11). Because cell migration involves protease activity and ADAM-15 is strongly expressed in human epithelial intestinal cells, we hypothesized that ADAM-15 might be involved in epithelial cell migration along intestinal crypts. To test this hypothesis, we performed a wound-healing assay using the ECIS technology (12, 13, 18) and using the Caco2-BBE cell line as a model system. Experiments were conducted as follows. Cells were seeded on plates containing gold-film electrodes through which elevated field pulses were applied and that also measure cell resistance. We first determined the ideal frequency used to measure resistance of Caco2-BBE cells. We performed resistance measurements during a frequency scan on ECIS 8W1E plates, first in the presence of confluent cells, and then without cells on naked electrodes as described in the MATERIALS AND METHODS. Figure 5A represents the profiles of resistances obtained for different frequencies in eight wells. The upper set of curves represents the measurements taken in the presence of confluent cells and the lower set of curves represents the measurements taken in the same wells after cell trypsinization. It is then possible to determine the ideal frequency as it is represented in Fig. 5B, using the ratio of the log of resistances with cells over the log of resistances without cells. The ideal frequency is the frequency for which the ratio reaches a peak. For Caco2-BBE cells, the ratio peak was found for a log [frequency (Hz)] of 2.7, corresponding to a frequency of 500 Hz (Fig. 6B). All the resistance measurements performed on Caco2-BBE cells, using the ECIS technology, were therefore performed at a frequency of 500 Hz.

To investigate the role of ADAM-15 in wound-healing of human epithelial intestinal cells, we performed wound-healing experiments with Caco2-BBE cells transfected either with the short isoform of ADAM-15 or with the vector alone. To assess ADAM-15 overexpression in transfected cells, we performed flow cytometry experiments 24 h after transfection. Figure 6A, inset, shows that as early as 24 h after transfection, cells transfected with ADAM-15 (Caco2-BBE/ADAM-15) start expressing a higher level of ADAM-15 compared with cells transfected with the vector only (Caco2-BBE/Vect).

For the wound-healing assays, Caco2-BBE/Vect and Caco2-BBE/ADAM-15 cells cultured on ECIS 8W1E plates were submitted to an elevated voltage pulse of 40 kHz frequency, 4.5 V amplitude, and 30-s duration, and resistance measurements were then performed. At the end of the experiments, i.e., 19 h after the elevated voltage pulse application, cells on electrodes were observed under a microscope and pictures were taken. As shown in Fig. 6A, the application of the high-field pulse led to a drastic drop of cell resistance. After the wound, the resistances of Caco2-BBE/ADAM-15 cells did not increase to reach the resistance values of control, nonwounded cells (Fig. 6A). By contrast, the resistances of Caco2-BBE/Vect cells increased over time and reached the resistance values of control, nonwounded cells (Fig. 6A). These results were in correlation with the observations made at the end of the

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**Fig. 4.** ADAM-15 is expressed in both apical and basolateral membranes and in the intracellular compartment in Caco2-BBE cells. A: confocal microscopic localization of ADAM-15 (green) in Caco2-BBE cell monolayers also stained with phalloidin (actin, red). a, horizontal (xy) section near the apical plasma membrane; b, horizontal (xy) section near the basolateral plasma membrane and vertical (zy) section of polarized Caco2-BBE monolayers stained with the anti-ADAM-15 ectodomain and phalloidin. CTRL, control. B: filter-grown Caco2-BBE monolayers were subjected to domain-specific biotinylation [apical (Apic.), basolateral (Basol.)] for 30 min followed by Western blot analysis of biotinylated proteins. Biotinylated proteins were subjected to 7.5% SDS-PAGE followed by transfer to nitrocellulose membrane. The blot was immunostained with rabbit anti-ADAM-15 antibody (raised against the cytoplasmic domain).
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Another interesting function of ADAM-15 involves the cytoplasmic domain, because it has been reported that it possesses motifs likely to be involved in signal transduction (19, 29, 31).

Our results have shown that Caco2-BBE cells express two isoforms of ADAM-15 protein: one long isoform, corresponding to the isoform deposited on the DNA database, and one short isoform presenting a 70-bp deletion resulting in a shorter and partly different cytoplasmic tail. It has been shown that the cytoplasmic tail of ADAM-15 binds with two SH3-containing proteins, endophilin I and SH3PX1 (17). Poghosyan et al. (29) have shown that two tyrosines (Y715 and Y735) within the cytoplasmic tail of ADAM-15 are phosphorylated and can bind with proteins of the Src family protein-tyrosine kinases. We have shown that the deletion leads to a different cytoplasmic tail that does not contain the predicted proline-rich domains present in the long cytoplasmic tail and for which the environment of tyrosine 735 is changed. It is interesting to speculate that each of the two different extensions of the ADAM-15 cytoplasmic tail represents a modular unit designed to interact with unidentified cytoplasmic or membrane proteins. It is likely that the different tails might interact differently with cytoskeletal elements or integrins or transduce different signal cascades to mediate different cell-specific functions. These results are in agreement with previous studies (32) showing that murine T lymphocytes and monocytes lines express three isoforms of ADAM-15. These isoforms differ in their cytoplasmic domain and present differential interaction among their cytoplasmic domains and Src family protein-tyrosine kinases (32).

Our results demonstrate that ADAM-15 protein is expressed in Caco2-BBE monolayers and in human epithelial intestinal cells in the intracellular compartment, as well as at the cell surface membrane. On the cell surface of Caco2-BBE monolayers, both pro-ADAM-15 and processed ADAM-15 can be detected, although both forms apparently only represent a small amount of the total ADAM-15 in the cell. However, the precursor found on the cell surface could be processed at a later stage after endocytosis (21). It has been suggested that ADAM-15 processing may require endocytosis from the cell surface (21). Interestingly, the major form of ADAM-15 detected by Western blot analysis found in colonic tissue seems to be different than the one found in the small intestine or in Caco2-BBE cells and might be explained by different degrees of glycosylation. Human ADAM-15 is the only ADAM with an RGD motif within the putative integrin-binding sites of desintegrin domains (19). The presence of the RGD domain is suggested to play a role in cell-cell adhesion (19). In addition, it is known that the RGD domain interacts with β2-integrins that are expressed in immune cells (27). Presence of a transmembrane protein that contains an RGD domain in intestinal epithelial cells may have some pathophysiological relevance. For example, during intestinal inflammation, leukocyte β2-integrins CD11b/CD18 and CD11c/CD18 are known to bind to the basolateral aspect of intestinal epithelial cells (27). The expression of the β2-counter receptor in intestinal epithelial cells might help neutrophils to bind to the epithelium. This scenario is possible, because we have shown that ADAM-15 protein is expressed in the basolateral membrane of Caco2-BBE monolayers and in human intestinal epithelial cells. However, on the basis of our in vitro and in vivo results, ADAM-15 is likely to be expressed to the apical aspect of intestinal epithelial. It is conceivable that the metalloprotease domain may play an important proteolytic activ-
activity against the lumen content. This function is supported by the fact that the mature, proteolytically active, ADAM-15 is the dominant form expressed in the apical plasma membrane of Caco2-BBE monolayers.

Colonic epithelial cells undergo cell cycle arrest, lineage-specific differentiation, and apoptosis as they migrate along the crypt axis to reach the luminal surface. The increase in extracellular matrix (ECM)-associated genes suggests that increased deposition of an ECM may be a function of differentiated colonic epithelial cells. Indeed, interaction of epithelial cells with ECM components is known to modulate differentiation (4). In the present study, we observed that ADAM-15 is expressed in human intestinal epithelial cells, suggesting that it may play a role in intestinal epithelial cell migration and differentiation. Because ADAM-15 possesses a protease activity (11, 22) and because cell migration processes involve protease activity, we investigated the role of ADAM-15 in intestinal epithelial cell migration. To examine this hypothesis, wound-healing experiments were performed by using the ECIS technology. The ECIS instrument allows performing automated and accurate wound-healing assays. The ECIS wound is very well defined, because it includes only cells grown on the 250-μm diameter electrode, i.e., an area of \( \sim 5 \times 10^{-4} \text{ cm}^2 \). In our system, overexpression of ADAM-15 in Caco2-BBE cells resulted in the inhibition of wound-healing processes. These results are consistent with the work of Herren et al. (16) who showed that ADAM-15 overexpression in the fibroblastic cell line NIH3T3 inhibited cell migration. The inhibition of wound repair we observed in Caco2-BBE overexpressing ADAM-15 could be due to an enhancement of cell-cell interactions as suggested by the work of Herren et al. (16) on NIH3T3 cells overexpressing ADAM-15. As has already been suggested (1), ADAM-15 could inhibit cell migration by interacting with integrins and therefore balancing interactions between integrins and matrix.

An impairment of the integrity of the mucosal epithelial barrier is observed in the course of various intestinal disorders including inflammatory bowel diseases, celiac disease, intestinal infections, and various other diseases. The integrity of the intestinal mucosal surface barrier is reestablished by resealing of the surface epithelium through epithelial cell migration, proliferation, and differentiation. Our results showing that ADAM-15 plays a role in wound healing therefore suggest that ADAM-15 might be involved in intestinal pathologies, such as inflammatory bowel diseases. In addition, the fact that ADAM-15 overexpression inhibits wound healing processes suggests that ADAM-15 could prevent inappropriate cell migration and therefore metastasis formation. For that matter, in vivo studies have shown that the recombinant disintegrin domain of ADAM-15 possesses an anti-metastatic activity (34).

Further studies about the status and the function of ADAM-15 in vivo are needed to confirm the validity of these in vitro findings. ADAM-15 expression has already been shown to be dysregulated in some pathologies. For example, ADAM-15 expression has been shown to be upregulated in atherosclerosis (1) as well as in osteoarthritic cartilage (6).

In this report, we show for the first time that 1) ADAM-15 protein is expressed in both native human intestinal epithelium as well as in human intestinal epithelial cell lines; 2) a human intestinal epithelial cell line expresses two isoforms of

Fig. 6. Overexpression of ADAM-15 impairs Caco2-BBE cell wound healing. After transfection of Caco2-BBE cells with the short isoform of ADAM-15 (Caco2-BBE/ADAM-15) or with the vector alone (Caco2-BBE/Vect), ADAM-15 expression was assessed by flow cytometry (A, inset). Confluent Caco2-BBE/ADAM-15 and Caco2-BBE/Vect cells, seeded in ECIS plates, were submitted (wounded) or not (ctl) to an elevated voltage pulse of 40 kHz frequency, 4.5 V amplitude, and 30-s duration. The wound was then allowed to heal from cells that did not undergo the elevated voltage pulse and that surround the small active electrode. A: resistance was measured before and after the elevated voltage pulse application as described in the MATERIALS AND METHODS. The measurements were stopped \(- 19 \text{ h after the wound.} \) B: at the end of the experiments, cells were washed twice in PBS, and pictures were taken of cells on the electrodes. Arrows indicate the direction of cell repair and migration.
ADAM-15 that differ in their cytoplasmic tail, suggesting differential signal transduction and therefore different roles; and J) ADAM-15 regulates wound-healing processes in human intestinal epithelial cells.

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