Caspase Proteolysis of the Integrin β4 Subunit Disrupts Hemidesmosome Assembly, Promotes Apoptosis, and Inhibits Cell Migration

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Caspases are a conserved family of cell death proteases that cleave intracellular substrates at Asp residues to modify their function and promote apoptosis. In this report we identify the integrin β4 subunit as a novel caspase substrate using an expression cloning strategy. Together with its α6 partner, α6β4 integrin anchors epithelial cells to the basement membrane at specialized adhesive structures known as hemidesmosomes and plays a critical role in diverse epithelial cell functions including cell survival and migration. We show that integrin β4 is cleaved by caspase-3 and -7 at a conserved Asp residue (Asp1109) in vitro and in epithelial cells undergoing apoptosis, resulting in the removal of most of its cytoplasmic tail. Caspase cleavage of integrin β4 produces two products, 1) a carboxy-terminal product that is unstable and rapidly degraded by the proteasome and 2) an amino-terminal cleavage product (amino acids 1–1109) that is unable to assemble into mature hemidesmosomes. We also demonstrate that caspase cleavage of integrin β4 sensitizes epithelial cells to apoptosis and inhibits cell migration. Taken together, we have identified a previously unrecognized proteolytic truncation of integrin β4 generated by caspases that disrupts key structural and functional properties of epithelial cells and promotes apoptosis.

Apoptosis is a genetically regulated cellular suicide program activated by diverse signals that results in a series of stereotypical events culminating in cell death. These events include membrane blebbing, nuclear and DNA fragmentation, loss of cell adhesion, dismantling of the cytoskeleton, and packaging of the nuclear and cytoplasmic remnants into apoptotic bodies which are engulfed and degraded by adjacent cells. Caspases are highly conserved cell death proteases that execute many of these stereotypical events by cleaving target proteins at aspartic acid residues (1). For example, caspases trigger the internucleosomal fragmentation of DNA by cleaving ICAD, an inhibitor of the caspase-activated DNase (CAD), thereby releasing ICAD from the inactive ICAD-CAD complex and activating CAD (2, 3). Caspases dismantle the nuclear envelope and intermediate filament cytoskeleton by specifically proteolyzing the nuclear lamins, cytokeratins 14, 18, and 19 in epithelial cells, vimentin in mesenchymal cells, and desmin in muscle cells (4–10). Caspases also disrupt the actin microfilament network by proteolyzing and activating the actin-severing protein gelsolin and by cleaving actin directly (11, 12). In addition, caspases proteolyze several components of adherens junctions and desmosomes, intercellular junctions attached to the actin microfilament or cytoskeleton intermediate filament networks, respectively, which play a critical role in cell-cell adhesion in epithelial tissues. Indeed, classical (E-cadherin) and desmosomal cadherins (desmoglein-1 and -3 and desmocollin-3), β-catenin, plakoglobin, and desmosomal plaque proteins (plakophilin-1, desmoplakin-1 and -2) are all caspase substrates (13–16). Clearly, adherens junctions and desmosomes and their associated cytoskeletal networks have been extensively targeted for degradation by caspases. The proteolytic disassembly of these structures by caspases likely contributes to the disruption of cell-cell adhesion and the dramatic cytoskeletal reorganization that typifies apoptosis.

In an effort to systematically decipher the molecular mechanisms by which caspases induce apoptosis, we have used a small pool expression cloning approach to isolate caspase substrates (9, 17–20). Here, we report the identification of the integrin β4 subunit as a novel caspase substrate. Integrins are a family of heterodimeric cell surface receptors composed of an α and β subunit that adhere cells to the extracellular matrix (ECM) and transmit signals from the ECM that regulate cell survival, proliferation, differentiation, and migration (21). The integrin β4 subunit is distinguished from other integrin subunits by virtue of several unique characteristics. In contrast to the short intracellular domains of other integrins, integrin β4 has a large cytoplasmic tail spanning ~1000 amino acids that consists of two pairs of fibronectin type III (FNIII) repeats separated by a connecting sequence (22, 23). Moreover, the integrin β4 subunit...
and its α6 partner (α6β4 integrin) are receptors for the extracellular matrix protein laminin-5, recently designated laminin-332 by a laminin nomenclature committee (24). α6β4 integrin is organized into multiprotein cytoplasmic plaques called hemidesmosomes that anchor cells in the basal layer of epithelial tissues, including skin and the mammary gland, to the basement membrane. Hemidesmosomes are attached to the cytoskeletal network, whereas other integrins are attached to actin microfilaments (22, 23). Additional protein components of hemidesmosomes are BP180 (BPAG2), a type II transmembrane protein, BP230 (BPAG1), and the linker protein plectin. Importantly, the cytoplasmic tail of integrin β4 plays a key role in the localization of α6β4 integrin to hemidesmosomal structures and in the recruitment of other hemidesmosomal proteins to these structures. Specifically, the first and/or second FNIII repeat and a portion of the connecting sequence are required for assembly of α6β4 integrin into hemidesmosomes and plectin binding, whereas the third FNIII repeat and part of the connecting sequence mediate BP180 binding (25–27). BP230 interacts with the third and fourth FNIII repeats and the carboxyl terminus of integrin β4 (28). The paramount importance of hemidesmosomes in maintaining the structural integrity of certain epithelial tissues is evident from patients with junctional epidermolysis bullosa (JEB) with congenital pyloric atresia, a fatal skin blistering disease characterized by abnormal hemidesmosomes, detachment of epithelial cells from the basement membrane, and mutations in the β4 or α6 subunit genes (29, 30). Indeed, mice with targeted deletion of the entire integrin β4 gene or the cytoplasmic domain lack hemidesmosomes and develop a JEB-like syndrome notable for increased detachment and death of epithelial cells (31–33). Moreover, deletion of integrin β4 or its carboxyl-terminal tail in keratinocytes results in profound abnormalities in cell migration and cell survival that likely impair wound healing (34, 35). Taken together, these findings point to a critical role for the cytoplasmic tail of integrin β4 in the structural integrity, function, and survival of certain epithelial tissues.

In this report we demonstrate for the first time that the integrin β4 subunit is cleaved by caspase-3 and -7 at Asp\textsuperscript{1109} in vitro and in cells undergoing apoptosis, thereby removing much of its cytoplasmic tail, including all four FNIII repeats. Caspase proteolysis of integrin β4 generates a carboxyl-terminal product that is unstable and rapidly degraded by the proteasome and an amino-terminal cleavage product that is unable to assemble into mature hemidesmosomes. In addition, caspase cleavage of integrin β4 sensitizes epithelial cells to apoptosis and inhibits cell migration. Collectively, our results point to a novel caspase-mediated truncation of integrin β4 that disrupts key structural and functional properties of epithelial cells and promotes apoptosis.

**EXPERIMENTAL PROCEDURES**

**Plasmid Constructs**—cDNAs encoding wild-type (WT) full-length integrin β4 or its cytoplasmic tail (amino acids 734–1752) were constructed by PCR amplifying the human integrin β4 cDNA using the primer pairs 5′-ccgctcggactATGGCAGGGCCACGCCCAAGCCCA-3′ and 5′-ccgctcggactTCAAGGTGGGAAGAAGTGTGGTGC-3′ (WT) or 5′-ccgctcggactATGGGGAAGAAGTGTGGTGC-3′ and 5′-ccgctcggactATGGGTGGGAAGAAGTGTGGTGC-3′ (tail). The PCR products were then digested with EcoRI and Xhol and subcloned into pcDNA3 (Invitrogen). A Δ1109E mutant full-length and tail β4 construct in which the putative caspase cleavage site at Asp\textsuperscript{1109} was replaced with a Glu residue were generated using the QuickChange site-directed mutagenesis kit (Stratagene) using the primers 5′-AGGCATGAACTGAGCGGAGGCTTCACAGAAGTCA-3′ and 5′-TGACTTGTAAGCTGCTCGCCGTTTATCTGGTGTCC-3′. cDNAs encoding carboxyl-terminal GFP-tagged, full-length integrin β4 (WT-β4) or the amino-terminal caspase cleavage fragment composed of amino acids 1–1109 (caspase-truncated β4, designated Tr-β4) were generated by PCR amplification of the wild-type human integrin β4 cDNA using the primers 5′-gccctcggactACCCGCTTCACACCTCCT-3′ and 5′-cccctcggactGCCGAGGATCGGAGACTGCTTTCCATCGC-3′ and 5′-gccctcggactGCCGAGGATCGGAGACTGCTTTCCATCGC-3′. cDNAs were verified by DNA sequence analysis.

**Small Pool Expression Cloning**—cDNAs encoding putative caspase substrates were isolated from a human prostate adenocarcinoma cDNA library (Invitrogen) by small pool expression cloning as described previously (9, 17–20).

**Caspase Cleavage of Integrin β4 in Vitro**—Full-length integrin β4, the WT integrin β4 cytoplasmic tail, or mutant Δ1109E integrin β4 tail were \textsuperscript{35}S-labeled with \textsuperscript{35}S-methionine using the TNT T7 Quick Coupled Transcription/Translation system (Promega). \textsuperscript{35}S-Labeled full-length integrin β4 was incubated with buffer or 2.5 ng of caspase-3 for 1 h at 37 °C, whereas the \textsuperscript{35}S-labeled tail proteins were incubated with buffer or 2.5 or 25 ng of caspase-1, -2, -3, -5, -6, -7, -8, -9 or -9 for 1 h at 37 °C; cleavage reactions were analyzed as described previously (19, 36).

**Cell Culture and Apoptosis Experiments**—Immortalized, non-transformed human MCF-10A mammary epithelial cells (37) and human MDA-MB-435 breast cancer cells were purchased from the ATCC. MCF-10A cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 100 μg/ml recombinant tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which was produced as described previously (39, 40), or 1 μM staurosporine (Sigma). To verify that the apoptotic cleavage of integrin β4 was mediated by caspases, cells were treated with 1 μM or 50 μM zVAD-fmk, a broad spectrum caspase inhibitor, and then

**REFERENCES**

- Immortalized integrin β4-deficient keratinocytes derived from a patient with JEB with pyloric atresia (34) were kindly provided by M. Peter Marinkovich, Stanford University School of Medicine. JEB keratinocytes were cultured in defined keratinocyte-serum-free medium (Invitrogen) and penicillin/streptomycin (Invitrogen). For MCF-10A cells, apoptosis was induced by addition of 1 μg/ml recombinant tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which was produced as described previously (39, 40), or 1 μM staurosporine (Sigma). To verify that the apoptotic cleavage of integrin β4 was mediated by caspases, cells were treated with 1 μM or 50 μM zVAD-fmk, a broad spectrum caspase inhibitor, and then

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A protein of ~75 kDa (denoted by an asterisk) in 35S-labeled protein pool 59 is cleaved by caspase-3 into a ~58-kDa fragment (indicated by an arrow); this fragment was not observed when protein pool 59 was incubated with buffer control. 35S-labeled protein pools were generated from small pools of a human prostate adenocarcinoma cDNA library and incubated with 2.5 ng of recombinant caspase-3 as described (9, 17–20). B, the cDNA encoding the ~75-kDa protein that is proteolyzed by caspase-3 into a ~58-kDa fragment was isolated from cDNA pool 59 by subdividing the pool and retesting smaller pools by the same approach. Clone 59A5 was sequenced and identified as a partial integrin \( \beta_4 \) subunit cDNA. C, the full-length integrin \( \beta_4 \) subunit is cleaved by caspase-3 in vitro. Full-length 35S-labeled integrin \( \beta_4 \) (denoted by an asterisk) is cleaved by 2.5 ng of caspase-3 into two products of ~130 and ~79 kDa (indicated by arrows).

**FIGURE 1. Identification of the integrin \( \beta_4 \) subunit as a novel caspase-3 substrate in vitro by small pool expression cloning.** A, a protein of ~75 kDa (denoted by an asterisk) in 35S-labeled protein pool 59 is cleaved by caspase-3 into a ~58-kDa fragment (indicated by an arrow); this fragment was not observed when protein pool 59 was incubated with buffer control. 35S-labeled protein pools were generated from small pools of a human prostate adenocarcinoma cDNA library and incubated with 2.5 ng of recombinant caspase-3 as described (9, 17–20). B, the cDNA encoding the ~75-kDa protein that is proteolyzed by caspase-3 into a ~58-kDa fragment was isolated from cDNA pool 59 by subdividing the pool and retesting smaller pools by the same approach. Clone 59A5 was sequenced and identified as a partial integrin \( \beta_4 \) subunit cDNA. C, the full-length integrin \( \beta_4 \) subunit is cleaved by caspase-3 in vitro. Full-length 35S-labeled integrin \( \beta_4 \) (denoted by an asterisk) is cleaved by 2.5 ng of caspase-3 into two products of ~130 and ~79 kDa (indicated by arrows).
wound assay) to increase the percentage of migrating cells for analysis.

RESULTS

Identification of the Integrin β4 Subunit as a Caspase-3 Substrate in Vitro by Small Pool Expression Cloning—We have previously described an expression cloning method to systematically identify cDNAs encoding putative caspase substrates from small pools of a cDNA library (9, 17–20). In these experiments caspase-3 was added to 35S-labeled protein pools derived from small cDNA pools (48 cDNAs in each pool) of a human prostate adenocarcinoma cDNA library (Invitrogen). Pools with protein bands that were cleaved by caspase-3 were identified. An ~75-kDa protein (Fig. 1A, indicated by an asterisk) in 35S-labeled pool 59 was proteolyzed by caspase-3 into a fragment of ~58 kDa (Fig. 1A, indicated by the arrow) in vitro. To identify the caspase-3 substrate corresponding to the ~75-kDa band in protein pool 59, we divided cDNA pool 59 into progressively smaller pools, 35S-labeled these pools, and incubated them with a caspase-3 pool. Importantly, 35S-labeled full-length integrin β4 (Fig. 1C, indicated by the asterisk) was proteolyzed by caspase-3 into products of ~130 and ~79 kDa (Fig. 1C, indicated by arrows). Taken together, these results indicate that the integrin β4 subunit is proteolyzed by caspase-3 at a single site in vitro.

The Cytoplasmic Tail of the Integrin β4 Subunit Is Cleaved at Asp1109 by Caspases-3 and -7 in Vitro—Because caspases are intracellular proteases that do not have access to the extracellular and transmembrane domains of integrin β4, we examined the sensitivity of the 35S-labeled cytoplasmic tail of integrin β4 (amino acids 734–1752) to a panel of recombinant caspases. Incubation of the 35S-labeled β4 cytoplasmic tail with caspase-3 generated prominent fragments of ~79 and ~43 kDa (Fig. 2A, indicated by arrows) and reduced the amount of the intact cytoplasmic tail (Fig. 2A, denoted by an asterisk). Caspase-7, which cleaves substrates at a similar DEXD motif as caspase-3 (46), produced the same sized fragments but to a lesser extent. A faint band corresponding to the ~43-kDa product was also observed when the 35S-labeled β4 cytoplasmic tail was incubated with caspase-8. However, caspases-1, -2, -5, -6, and -9 did not proteolyze the β4 cytoplasmic tail. Of note, the observed ~79-kDa product was similar in size to the fragment produced by caspase-3 cleavage of full-length 35S-labeled β4 (Fig. 1C). These results indicate that the cytoplasmic tail of integrin β4 is preferentially proteolyzed by caspase-3 and -7 in vitro.

A potential caspase-3 and -7 cleavage motif (DEXD) (46) was identified in the β4 cytoplasmic tail (DELD1109↓R) that would be expected to yield a ~79-kDa carboxyl-terminal cleavage product. To determine whether integrin β4 was indeed cleaved by caspase-3 and -7 at this site in vitro, we replaced Asp1109 with a Glu residue and tested the sensitivity of the mutant D1109E β4 cytoplasmic tail to caspase proteolysis. Unlike the WT β4 cytoplasmic tail (Fig. 2A), the 35S-labeled D1109E β4 tail was not cleaved by caspase-3 or -7 (Fig. 2B), indicating that Asp1109 is the bona fide caspase-3 and -7 cleavage site in vitro. A domain map of the integrin β4 protein reveals that caspase proteolysis at Asp1109 removes much of the cytoplasmic tail, including all four fibronectin type III repeats and the connecting sequence (Fig. 2C). Taken together, these findings indicate that β4 integrin is cleaved by caspases-3 and -7 at Asp1109 in its cytoplasmic tail, thereby removing several domains involved in hemidesmosome assembly and/or signaling.

Integrin β4 Is Proteolyzed at Asp1109 by Caspases in Cells Undergoing Apoptosis and Produces an Unstable Cleavage Product That Is Degraded by the Proteasome—We next examined whether the integrin β4 subunit is cleaved in epithelial cells undergoing apoptosis. For these experiments we treated MCF-10A mammary epithelial cells, which express integrin β4 and assemble mature hemidesmosomes (47, 48), with 1 μg/ml TRAIL for 0–8 h. The amount of full-length integrin β4

FIGURE 2. The cytoplasmic tail of the integrin β4 subunit is cleaved at Asp1109 by caspases-3 and -7 in vitro. A, the 35S-labeled cytoplasmic tail of integrin β4 (denoted by an asterisk) is preferentially cleaved by caspases-3 and -7 into a ~79- and a ~43-kDa product (indicated by arrows) in vitro. The 35S-labeled integrin β4 cytoplasmic tail was incubated with buffer control or 2.5 or 25 ng of caspase-1, -2, -3, -5, -6, -7, -8, or -9 (C1-C9) for 1 h at 37 °C. The reaction products were separated by SDS-PAGE and detected by autoradiography. B, a mutant integrin β4 cytoplasmic tail in which Asp1109 is replaced with a Glu residue (D1109E) is resistant to caspase-3 proteolysis in vitro. The 35S-labeled mutant D1109E integrin β4 cytoplasmic tail was incubated with buffer control or 2.5 or 25 ng of caspase-1, -2, -3, -5, -6, -7, -8, or -9 (C1-C9) for 1 h at 37 °C. C, a protein domain map of the integrin β4 subunit showing the caspase cleavage site DELD1109↓R in the cytoplasmic domain.
Caspase Cleavage of Integrin β4

![Graphs and Images](Figure 3)

**FIGURE 3.** Integrin β4 is proteolyzed at Asp\(^{1109}\) by caspases in cells undergoing apoptosis and produces an unstable cleavage product which is degraded by the ubiquitin-proteasome system. A, full-length integrin β4 is rapidly degraded in human MCF-10A mammary epithelial cells treated with TRAIL (top panel) by a caspase-dependent mechanism. MCF-10A cells were treated with 1 μg/ml TRAIL or 1 μM staurosporine for 0–8 h. For the caspase inhibitor experiments, MCF-10A cells were preincubated for 1 h with 50 μM zVAD-fmk, a pan-caspase inhibitor, and then treated with 1 μg/ml TRAIL or 1 μM staurosporine for 4 h. Immunoblotting was performed with antibodies recognizing the cytoplasmic tail of integrin β4, protein kinase Cβ (PKCβ) or tubulin. The percentage of apoptotic nuclei was determined in parallel experiments. B, the apoptotic integrin β4 caspase cleavage product is degraded by the proteasome. MCF-10A cells were preincubated with 20 μM ZVAD-fmk, 100 nM epoxomicin (Epox), a proteasome inhibitor, or both for 1 h and then treated with 1 μg/ml TRAIL (or untreated) for 6 h. C, mutant D1109E integrin β4 is not cleaved during apoptosis. MDA-MB-435 breast cancer cells (which lack endogenous integrin β4 subunit) were transiently transfected with either WT or mutant D1109E integrin β4 cDNA. Twenty-four hours after transfection cells were treated with 0.5 μg/ml TRAIL and 1 μg/ml cycloheximide. In B and C, full-length integrin β4 (asterisks) and the ~72-kDa cleavage product (arrow) are indicated.

**Detecting Integrin β4 Cleavage.**—Integrin β4 was detected with an antibody directed against its carboxyl terminus. A decrease in the detectable full-length β4 at 8 h after TRAIL treatment (Fig. 3A, left panel) was noted. Although no β4 cleavage product was observed (data not shown), the reduction in the amount of full-length β4 in response to TRAIL was completely suppressed by the broad spectrum caspase inhibitor zVAD-fmk, suggesting that caspases were responsible for the observed reduction in full-length β4. The caspase substrate protein kinase Cβ was used as a positive control; it was cleaved into its expected size apoptotic fragment (49) within 2 h of TRAIL treatment, and its apoptotic proteolysis was blocked by zVAD-fmk. A reduction in full-length β4 was also observed in MCF-10A cells treated with staurosporine, and this reduction was suppressed by zVAD-fmk (Fig. 3A, right panel). These data suggest that caspases cleave the integrin β4 subunit during apoptosis in vivo and produce an unstable proteolytic product. Indeed, caspase proteolysis of several proteins has been demonstrated to yield unstable cleavage products that are rapidly degraded by the proteasome (50–53). Hence, we postulated that the integrin β4 carboxyl-terminal cleavage product was similarly degraded. Consistent with this hypothesis, a ~72-kDa β4 cleavage product was observed in TRAIL-treated MCF-10A cells preincubated for 1 h with 100 nM epoxomicin, a proteasome inhibitor (Fig. 3B). This proteolytic product is similar in size to the ~79-kDa product; some cleavage was also evident at t = 0 due to the toxicity of the transfection reagents (a prominent more rapidly migrating band that was also detected by the carboxyl-terminal integrin β4 Ab was also cleaved). Of note, in these transiently transfected cells, the ~72-kDa caspase cleavage product was detectable in the absence of proteasome inhibition, perhaps due to the high levels of the ectopically expressed β4. Conversely, D1109E-β4 was resistant to proteolysis with little production of the ~72-kDa fragment in TRAIL-treated cells. These results indicate that integrin β4 is cleaved by caspases at Asp\(^{1109}\) in cells undergoing apoptosis.

**Caspase Cleavage of Integrin β4 Disrupts Hemidesmosome Assembly.**—Because caspase cleavage of β4 integrin removes cytoplasmic domains required for its localization to hemidesmosomes and its binding to other hemidesmosomal proteins (25–27), we examined the ability of GFP-tagged full-length WT-β4 and caspase-truncated β4 (amino acids 1–1109, Tr-β4) to assemble into hemidesmosomes in integrin β4-deficient keratinocytes. These keratinocytes were derived from a patient with JEB and contain all of the protein components of hemidesmosomes except the integrin β4 subunit (34). Importantly, the addition of GFP to the carboxyl terminus of WT integrin β4 does not alter its ligand binding, localization to hemidesmosomes, or signaling (54–56). JEB keratinocytes stably expressing GFP WT-β4, GFP Tr-β4, or control-GFP vector were gen-
more diffusely distributed and was present in polarized basal structures in only 44 ± 2.8% of cells. These results indicate that although Tr-β4 is expressed on the cell surface, it often fails to incorporate into hemidesmosome-like structures.

We next examined whether WT-β4 or Tr-β4, when localized in polarized basal structures, supported the assembly of mature hemidesmosomes by recruiting BP180 and plectin to these hemidesmosome-like structures. In JEB keratinocytes stably expressing WT-β4, polarized basal WT-β4 colocalized with BP180 in 92 ± 4.2% of cells (Fig. 5A) and plectin in 98 ± 1.4% of cells (Fig. 5B). In contrast, polarized basal Tr-β4 colocalized with BP180 in only 53 ± 5.7% of cells (Fig. 5A) and rarely colocalized with plectin (only 1.5 ± 0.7% of cells, Fig. 5B). Collectively, these data indicate that caspase cleavage of integrin β4 disrupts the assembly of mature hemidesmosomes.

**Caspase Cleavage of Integrin β4 Promotes Apoptosis**—To determine whether caspase cleavage of integrin β4 alters the sensitivity of epithelial cells to apoptosis induction, we treated JEB pools stably expressing GFP vector or GFP-tagged WT-β4, D1109E-β4, or Tr-β4 with 5 μg/ml TRAIL for 0–8 h. Both Tr-β4 and WT-β4, but not cleavage-resistant D1109E-β4, sensitized JEB keratinocytes to TRAIL-induced apoptosis 2 and 4 h after treatment (Fig. 6). However, by 8 h the induction of apoptosis was similar in each of the JEB pools. WT-β4 was cleaved by caspases as early as 2 h after TRAIL treatment (data not shown). These results indicate that caspase cleavage of integrin β4 sensitizes keratinocytes to TRAIL-induced apoptosis.

**Caspase Cleavage of Integrin β4 Inhibits Keratinocyte Migration**—Because caspase-3 has been implicated in cell migration in non-apoptotic epithelial cells (57), we examined whether integrin β4 was cleaved in migrating keratinocytes. To this end, confluent JEB cells stably expressing WT-β4 were scraped with a pipette tip in a grid-like pattern (multi-scratch wound assay). A reduction in the amount of full-length WT-β4 was observed at 48 h in this assay, and this reduction was inhibited by zVAD-fmk (Fig. 7A). These results indicate that integrin β4 is cleaved by caspases in migrating...
keratinocytes. To determine the effects of integrin β4 cleavage on cell migration, confluent JEB cells stably expressing GFP vector or GFP-tagged WT-β4 or Tr-β4 were scraped with a pipette tip, and wound closure was measured 48 h later. Although WT-β4 cells were highly motile in this assay, Tr-β4 cells exhibited little if any migration and were not significantly different from β4-deficient vector-transduced JEB cells (Fig. 7, B and C). Importantly, these migration differences were not due to differences in cell number (data not shown). These results suggest that caspase cleavage of integrin β4 inhibits keratinocyte migration.

**DISCUSSION**

We have demonstrated that the integrin β4 subunit is a novel caspase substrate that is cleaved by caspases-3 and -7 in vitro and in apoptotic epithelial cells. Caspases-3 and -7 proteolyze integrin β4 at a DEXD consensus caspase-3 and -7 cleavage motif (DELD-DED1109-D, in its cytoplasmic tail. Interestingly, this aspartic acid residue and the entire cleavage motif in integrin β4 has been highly conserved during evolution (DELD-R in dogs and cattle and DETD-R in mice and rats), suggesting that caspase proteolysis of β4 may occur at this same site during apoptosis in other species as well. As illustrated in Fig. 2C, caspase cleavage of β4 removes much of its cytoplasmic tail, including all four of the FNIII repeats and the connecting sequence. The resulting carboxy-terminal caspase cleavage product is unstable and is rapidly degraded by the proteasome. Indeed, the amino-terminal amino acid of this cleavage fragment (Arg1110) is a destabilizing residue that likely targets this cleavage product for degradation by the ubiquitin-dependent N-end rule pathway (58). Caspase proteolysis of NF-κB and the Drosophila inhibitor of apoptosis protein DIAPI also generate unstable cleavage products that are degraded by the N-end rule pathway.
in plectin recruitment to hemidesmosomal structures by Tr-β4 compared with BP180 is that BP180 is a membrane spanning protein that also interacts with α6 integrin and laminin-5 (59–61). Hence, α6 integrin and laminin-5 may contribute to BP180 recruitment to hemidesmosomes even in the absence of the cytoplasmic tail of β4. In contrast, plectin is dependent on the first and/or second FNIII repeat and a portion of the connecting sequence of β4 (26, 27), domains that are absent from Tr-β4, for incorporation into hemidesmosomes. Collectively, our results suggest that caspase cleavage of integrin β4 disrupts mature hemidesmosomes, an event that may promote the detachment of epithelial cells from the basement membrane and subsequent extracellular matrix detachment-induced apoptosis or anoikis.

Consistent with this idea, we have shown that caspase cleavage of integrin β4 sensitizes keratinocytes to apoptosis. Specifically, we demonstrated that JEB cells stably expressing Tr-β4 or WT-β4 (which was cleaved in response to TRAIL treatment), but not caspase cleavage-resistant D1109E-β4, were more sensitive to apoptosis at early time points after treatment with TRAIL. These findings are concordant with reports that deletion of various domains in the cytoplasmic tail of integrin β4 result in enhanced apoptosis in keratinocytes and mammary epithelial cells (35, 62, 63). Because the cytoplasmic tail of integrin β4 is required for laminin-5-induced activation of cell survival pathways such as NF-κB and phosphatidylinositol 3-kinase (35, 62, 63), proteolytic removal of this domain by caspases might promote apoptosis by abrogating these survival signals.

In addition, we observed that integrin β4 is cleaved by caspases in migrating keratinocytes during wound healing, a finding that is consistent with a recent report implicating caspase-3 in cell migration of non-apoptotic epithelial cells (57). We have also demonstrated that proteolysis of integrin β4 has important functional consequences for cell migration. Although caspase-truncated β4 was unable to promote keratinocyte migration in a wound closure assay, full-length β4 robustly enhanced cell motility. These results indicate that caspase cleavage of integrin β4 inhibits cell migration. Our results are in agreement with previous reports demonstrating that integrin β4-deficient JEB keratinocytes identical to those used in these experiments or keratinocytes expressing a less extensively truncated β4 (amino acids 1–1355) are impaired in cell migration, an important component of wound healing (34, 35). These defects in cell migration have been attributed to impaired Rac1, NF-κB, and/or c-Jun NH2-terminal kinase activation in β4-deficient keratinocytes or keratinocytes expressing truncated β4. Indeed, it seems likely that caspase cleavage of integrin β4 alters its downstream signaling to these and other pathways by removing key signaling residues/domains, an hypothesis that will be systematically explored in future studies. Collectively, the findings presented here demonstrate a novel proteolytic mechanism that profoundly alters integrin β4 localization and function. Together with the recent demonstration that the intracellular domains of other receptors such as epidermal growth factor receptor and HER-2/ErbB2 are cleaved by caspases (64–66), these findings suggest that

FIGURE 7. Caspase cleavage of integrin β4 inhibits keratinocyte migration. A, immunoblot analysis of JEB cells stably expressing GFP-tagged WT-β4 that were subjected to a multi-scratch wound assay as described under “Experimental Procedures” in the absence or presence of 50 μM ZVAD-fmk. Lysates were prepared 0–48 h after scratching. B, photomicrograph of a wound closure experiment. Confluent JEB cells stably expressing GFP vector or GFP-tagged WT-β4 or Tr-β4 were scraped with a pipette tip, and wound closure was assessed at 48 h as described under “Experimental Procedures.” C, data are presented as the mean ± S.E. of three experiments. *p < 0.05 versus vector control.

(51, 52), suggesting that caspases and the ubiquitin-proteasome pathway may collaborate to efficiently degrade certain substrates during the execution of apoptosis.

We have also demonstrated that removal of the cytoplasmic tail of integrin β4 by caspases, resulting in a truncated β4 integrin (amino acids 1–1109), has important structural and functional consequences. Although arbitrary deletional analyses of the cytoplasmic tail of β4 have demonstrated its importance in hemidesmosome assembly and diverse functions (25–27, 33, 35), our results provide the first evidence that β4 indeed undergoes proteolytic removal of its cytoplasmic tail in vivo during a normal cellular process, namely, apoptosis. Regarding the structural consequences, caspase-truncated β4, unlike full-length β4, is unable to assemble mature hemidesmosomal structures, which play a critical role in adhering epithelial cells to the basement membrane. Specifically, we have demonstrated that although Tr-β4 is present on the cell surface, it is often diffusely distributed rather than localized in polarized basal structures characteristic of hemidesmosomes. We also observed that Tr-β4 was completely impaired in its ability to recruit the hemidesmosomal protein plectin to hemidesmosome-like structures, whereas BP180 recruitment was partly impaired. One potential explanation for the more severe defect

Caspase Cleavage of Integrin β4
Caspase Cleavage of Integrin \( \beta4 \)

caspases may participate more broadly in regulating receptor-mediated signaling.

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REFERENCES

1. Niessen, C. M., Hulsman, E. H., Oomen, L. C., Kuikman, I., and Sonnenberg, A. (1998) *Nature* **391**, 96–99.
2. Liu, X., Zou, H., Slaughter, C., and Wang, X. (1997) *Cell* **89**, 175–184.
3. Lazebnik, Y. A., Takahashi, A., Moir, R. D., Goldman, R. D., Poirier, G. G., Kaufmann, S. H., and Earnshaw, W. C. (1995) *Proc Natl Acad Sci U S A* **92**, 9042–9046.
4. Rao, L., Perez, D., and White, E. (1996) *Cell Biol. Cell Biol.* **135**, 1441–1455.
5. Caulin, C., Salvesen, G. S., and Oshima, R. G. (1997) *Science* **278**, 6848–6853.
6. Kayalar, C., Ord, T., Testa, M. P., Zhong, L.-T., and Bredesen, D. E. (1996) *J Biol Chem.* **271**, 16775–16781.
7. Ku, N.-O., Liao, J., and Omary, M. B. (1997) *J Biol Chem.* **272**, 33197–33203.
8. Steinhausen, U., Weiske, J., Badock, V., Tauber, R., Bommer, K., and Huber, O. (2001) *J Biol Chem.* **276**, 4972–4980.
9. Weiske, J., Schoneberg, T., Schroder, W., Hatzfeld, M., Tauber, R., and Huber, O. (2001) *J Biol Chem.* **276**, 41175–41181.
10. Herren, B., Levkau, B., Raines, E. W., and Ross, R. (1998) *Cell Biol. Cell Biol.* **19**, 1589–1601.
11. Dusek, R. L., Getsios, S., Chen, F., Park, J. K., Amargo, E. V., Cryns, V. L., and Green, K. J. (2006) *J Biol Chem.* **281**, 3614–3624.
12. Chenn, F., Arseniev, O. K., and Cryns, V. L. (2001) *J Biol Chem.* **276**, 27542–27548.
13. Chenn, F., Kamradt, M., Mulcahy, M., Byun, Y., Xu, H., McKay, M. J., and Cryns, V. L. (2002) *J Biol Chem.* **277**, 16775–16781.
14. Miyoshi, Y., Nara, T. A., Peter, E. P., Raper, D. M., Meier, P., Garcia-Calvo, M., Houltzager, V. M., Nordstrom, P. A., Roy, S., Vaillancourt, J. C., and Earnshaw, W. C. (1995) *J Biol Chem.* **270**, 17907–17911.
15. Stahl, S., Weitzman, S., and Jones, C. J. (1997) *J Cell Sci.* **110**, 56–53.
16. McPhee, R. J., and Jones, C. J. (2001) *J Cell Sci.* **114**, 4197–4206.
17. Emoto, Y., Manohe, Y., Steinhardt, G., Kisaki, H., Kharbanda, S., Robertson, M., Ghayur, T., Wong, W. W., Kamen, R., Weichselbaum, R., and Kufe, D. (1995) *EMBO J.* **14**, 6148–6156.
18. Breitschopf, K., Kaender, J., Malchow, P., Zeiher, A. M., and Dimmeler, S. (2000) *Cell Biol. Cell Biol.* **120**, 1886–1896.
19. Meier, P., Wilson, R., Tenev, T., Zachariou, A., Pieters, A., Deas, E., and Meier, P. (2003) *Nat Cell Biol.* **5**, 467–473.
20. Rathore, N., Matta, H., and Chaudhary, P. M. (2004) *J Biol Chem.* **279**, 39358–39365.
21. Demontis, S., Rigo, C., Piccinini, S., Mazzuz, M., Sonego, M., Fabris, M., Brancolini, C., and Maestro, R. (2006) *Cell Death Differ.* **13**, 335–345.
22. Geuijen, C. A., and Sonnenberg, A. (2002) *Mol Cell Biol.* **12**, 3845–3858.
23. Tsuruta, D., Hopkinson, S. B., and Jones, C. J. (2003) *Cell Motil. Cytoskeleton* **54**, 122–134.
24. Tsuruta, D., Hopkinson, S. B., Lane, K. D., Werner, M. E., and Cryns, V. L., and Jones, C. J. (2003) *J Biol Chem.* **278**, 38707–38714.
25. Zhao, W., Wang, D., Zhao, Z., Xiao, Y., Sengupta, S., Zhang, R., Lauber, K., Wesselingh, S., Feng, L., Rose, T. M., Shen, Y., Zhang, J., Prestwich, G., and Xu, Y. (2006) *J Biol Chem.* **281**, 29357–29368.
26. Varshavsky, A. (2003) *Nat Cell Biol.* **5**, 373–376.
59. Hopkinson, S. B., Baker, S. E., and Jones, J. C. (1995) J. Cell Biol. 130, 117–125
60. Hopkinson, S. B., Findlay, K., deHart, G. W., and Jones, J. C. (1998) J. Investig. Dermatol. 111, 1015–1022
61. Tasanen, K., Tunggal, L., Chometon, G., Bruckner-Tuderman, L., and Aumailley, M. (2004) Am. J. Pathol. 164, 2027–2038
62. Weaver, V. M., Lelievre, S., Lakins, J. N., Chrenek, M. A., Jones, J. C., Giancotti, F., Werb, Z., and Bissell, M. J. (2002) Cancer Cell 2, 205–216
63. Zahir, N., Lakins, J. N., Russell, A., Ming, W., Chatterjee, C., Rozenberg, G. I., Marinkovich, M. P., and Weaver, V. M. (2003) J. Cell Biol. 163, 1397–1407
64. Tikhomirov, O., and Carpenter, G. (2001) J. Biol. Chem. 276, 33675–33680
65. Benoit, V., Chariot, A., Delacroix, L., Deregowski, V., Jacobs, N., Merville, M. P., and Bours, V. (2004) Cancer Res. 64, 2684–2691
66. He, Y. Y., Huang, J. L., and Chignell, C. F. (2006) Oncogene 25, 1521–1531