Cleavage of the Cytoplasmic Domain of the Integrin $\beta_3$ Subunit during Endothelial Cell Apoptosis*

(Received for publication, October 20, 1997, and in revised form, April 30, 1998)

Jere Meredith, Jr.*, Zhaoiui Mu**, Takaomi Saido**, and Xiaoping Du***

From the *Department of Pharmacology, College of Medicine, University of Illinois, Chicago, Illinois 60612, the **Department of Vascular Biology, The Scripps Research Institute, La Jolla, California 92037, and the ***Laboratory for Prognetic Neuroscience, RIKEN Brain Science Institute, Saitama 351-0198, Japan

In this study, we report that the cytoplasmic domain of the integrin $\beta_3$ subunit is a target for limited proteolysis during apoptosis of human umbilical vein endothelial cells. Calpain inhibitors inhibited the cleavage of the $\beta_3$ cytoplasmic domain, indicating that calpain is required. Calpain-mediated proteolysis of fodrin was also detected, indicating that calpain is activated during endothelial cell apoptosis. A phosphatase inhibitor, sodium orthovanadate, inhibited endothelial cell apoptosis and cleavage of fodrin, suggesting that protein dephosphorylation preceded integrin cleavage in the apoptosis signaling pathway. $\beta_3$ cleavage was observed in cells that were viable, suggesting that it is an early event and not the consequence of post-death proteolysis. The extent of $\beta_3$ cleavage correlated with a loss in the capacity of cells to reattach to matrix proteins. Loss of reattachment capacity during apoptosis was significantly retarded by a calpain inhibitor. As the $\beta_3$ cytoplasmic domain is required for integrin signaling and interaction with the cytoskeleton, our results suggest that cleavage in the $\beta_3$ cytoplasmic domain by calpain or a calpain-like protease negatively regulates integrin-mediated adhesion, signaling, and cytoskeleton association.

Programmed cell death or apoptosis is a cell suicide pathway involved in a variety of physiological and pathological events such as tissue morphogenesis, development, cancer, and neurodegenerative disorders (reviewed in Refs. 1 and 2). The mechanism of programmed cell death involves the activation of various intracellular proteases (reviewed in Ref. 3). The caspase family of proteases appears to play a key role in the signaling pathway (3, 4). In addition to caspases, the Ca$^{2+}$-dependent neutral protease, calpain, was also found to be activated during apoptosis in T cells and to cleave fodrin during apoptosis (3, 5–8).

Although the role of calpain during apoptosis is not clear, its substrates include several important intracellular signaling and cytoskeletal proteins, particularly those associated with integrin-focal adhesion complexes, such as focal adhesion kinase (pp125$\text{faK}$) (9), pp60$\text{src}$ (10, 11), fodrin (12), filamin and talin (13). In addition, we have recently found that calpain cleaves the cytoplasmic domain of the integrin $\beta_3$ subunit, and that calpain cleavage sites flank two NXYX motifs in the $\beta_3$ cytoplasmic domain required for integrin function (14).

Integrins are a family of cell adhesion receptors, each of which is a heterodimer complex of two transmembrane subunits, $\alpha$ and $\beta$ (reviewed in Ref. 15). Integrins bind to extracellular matrix adhesion proteins such as vitronectin, fibronectin, collagen, and laminin. Ligand binding to integrins transduces signals across the plasma membrane, resulting in activation of protein kinases, influx of calcium, elevation of intracellular pH, and hydrolysis of membrane phospholipids and reorganization of the cytoskeleton (reviewed in Ref. 16). Integrin-mediated signals are required for the survival of anchorage-dependent cells including endothelial and epithelial cells (17–21). Integrins interact with cytoskeletal proteins at focal adhesion sites and are critical for cytoskeletal organization and morphological characteristics of anchorage-dependent cells (22). Cytoskeleton reorganization and morphological changes such as membrane blebbing occur during apoptosis (3).

To determine if and how integrins are regulated during apoptosis, we investigated the possible relationship between intracellular proteases and integrins. We report here that the cytoplasmic domain of the integrin $\beta_3$ subunit is cleaved during endothelial cell apoptosis and that calpain is likely to be the protease involved. Since the integrin $\beta$-subunit cytoplasmic domain plays an important role in integrin-mediated cell adhesion and signaling (23–34), its proteolysis may block integrin-mediated signals and disrupt cytoskeleton organization during apoptosis.

EXPERIMENTAL PROCEDURES

Materials—Rabbit anti-peptide antibody, anti-5IC, is directed against the C-terminal 20 amino acid residues of the $\beta_3$ cytoplasmic domain. Rabbit antibody anti-5CS was produced using purified $\beta_3$ subunit as an immunogen and is directed against the extracellular domain. These antibodies have been described previously (14). Calpain inhibitors E64 and calpain inhibitor I were purchased from Boehringer Mannheim. The cell-permeable calpain inhibitor E64d was purchased from Sigma, and the antibody specific for calpain-cleaved fodrin was described previously (12).

Cell Culture—Human umbilical vein endothelial cells (HUVECs) were grown and maintained in endothelial cell growth medium (EGM, Clonetics) supplemented with an additional 8% fetal bovine serum (Life Technologies, Inc.). Cells were used between passages 2 and 10.

HUVECs were serum-starved by incubation in serum-free medium containing M199 (Life Technologies, Inc.), 0.1% bovine serum albumin (nuclease- and protease-free; Calbiochem), and insulin, selenium, and transferrin (GMS-G; Life Technologies, Inc.). For incubation in suspension culture, cells were detached with 0.01 mM Na$_2$HPO$_4$, 0.15 mM NaCl, 2.5 mM EDTA, pH 7.4 (PBS/EDTA) and resuspended in EGM containing...
0.5% methylcellulose (Sigma). Cells were plated on tissue culture dishes coated with 2% agarose (Sigma) in M199 medium to prevent attachment (17).

For reattachment experiments, cells incubated in suspension for 12 h were isolated by centrifugation, washed with PBS/EDTA for 5 min at 37 °C and then re-plated on tissue culture dishes in EGM. Cells were allowed to adhere and spread for 30 min. Adherent cells were lysed directly on the dish or were detached by PBS/EDTA and quantitated by cell counts prior to lysis. Unattached cells were collected by centrifugation. Both adherent and unattached cells were solubilized in buffer as described below and analyzed by immunoblotting.

**Immunoblotting**—Cells were isolated and washed twice with PBS/EDTA; for serum-starved cells, adherent cells were detached by incubation with PBS/EDTA and then recombined with the floating cells prior to lysis. Cells were lysed by incubation in 1% Triton X-100, 100 mM Tris, pH 7.4, 150 mM NaCl, 10 mM EDTA, 0.1 mM E64, and 1 mM phenylmethylsulfonyl fluoride for 10 min on ice. Lysates were cleared by centrifugation at 14,000 × g for 5 min. Protein concentrations of cell lysates were determined by BCA assay (Pierce). Equivalent amounts of cell lysates were analyzed by SDS-polyacrylamide gel electrophoresis followed by Western blotting either with antibodies, anti-β3C, against the C-terminal region of the β3 cytoplasmic domain or with the anti-β3 antibody. Reactions with antibodies were visualized using an enhanced chemiluminescence kit (Amersham Pharmacia Biotech), and Kodak X-Omat AR film. The immunoblots were scanned, and the uncalibrated optical density of each band was quantitated using NIH Image.

**Terminal Deoxynucleotidyltransferase-mediated Fluorescein-dUTP Nick End Labeling (TUNEL)**—Cells in suspension were isolated and incubated in PBS/EDTA for 5 min at 37 °C prior to fixation in 2% formaldehyde (methanol-free; Polysciences)/PBS for 15 min at room temperature. Following fixation, cells were washed twice with PBS and then extracted in 0.1% Triton X-100, 0.1% trisodium citrate for 2 min on ice. Cells were washed once with PBS and then incubated in 0.1% bovine serum albumin/PBS for 10 min at room temperature. Cells were labeled by incubation with terminal deoxynucleotidyltransferase and fluorescein-dUTP (Boehringer Mannheim) for 1 h at 37 °C. Labeled cells were washed with PBS and analyzed by flow cytometry (FACScan).

**RESULTS**

**Loss of the Cytoplasmic Domain of the Integrin β3 subunit during Suspension-induced Apoptosis**—To investigate the possibility that proteolysis may regulate integrin function during apoptosis, HUVECs were induced to undergo apoptosis by incubation in suspension. We and others have shown previously that endothelial cells undergo apoptosis when cultured in suspension which is characterized by DNA fragmentation and apoptotic morphology (17, 18). In agreement with these previous results, cells in suspension showed characteristic apoptotic morphology (Fig. 1), and DNA fragmentation (Fig. 2). Cells in suspension began to undergo apoptosis after 6 h of incubation, as demonstrated by labeling of fragmented DNA (Fig. 2A). The percentage of apoptotic cells increased with increasing time in suspension (Fig. 2A). Cells incubated in suspension for various lengths of time were solubilized in the presence of protease inhibitors. Equal amounts of lysates were then immunoblotted with anti-β3 antibodies. Two different antibodies were used; the antibody anti-β3 recognizes epitopes in the extracellular domain, while the antibody anti-β3C recognizes epitopes located in the C-terminal 20 residues of the cytoplasmic domain (14). Expression of the extracellular domain epitopes is essentially constant over the time course (anti-β3, Fig. 2, B and C). However, expression of the cytoplasmic domain epitopes significantly decreased with increasing lengths of time in suspension (anti-β3C, Fig. 2, B and C). These results indicate that the C-terminal domain is lost from a population of β3 subunits as a result of suspension culture. The time course of this loss of immunoreactivity with anti-β3C paralleled the time course of DNA fragmentation (Fig. 2, compare A and B), suggesting that this effect may be linked to the process of apoptosis.

**Cleavage of the Endothelial β3 Cytoplasmic Domain by Calpain**—We previously showed that calpain cleaves the cytoplasmic domain of the integrin β3 subunit during platelet aggrega-
Calpain inhibitor E64d inhibits cleavage of the cytoplasmic domain of the integrin β3 subunit. HUVECs were cultured in suspension for increasing lengths of time in the absence (−E64d) or presence of 20 μM E64d (+E64d). Cells were then solubilized and equivalent amounts of lysates were analyzed by Western blots with anti-β3C (γC). Optical density was quantitated by scanning and analysis with NIH Image. Relative amounts in loading were estimated by Western blot with anti-β3. Relative reactivities are expressed as a percentage of the antibody reaction at the 0 time point (corrected by the loading factor).

Calpain Cleavage of Fodrin during Endothelial Cell Apoptosis—To verify whether calpain is activated during apoptosis of T cells (5–7), we investigated whether calpain may be responsible for the loss in endothelial β3 cytoplasmic domain immunoreactivity. HUVECs were incubated in suspension for various lengths of time in the absence or presence of the membrane-permeable calpain inhibitor E64d. 20 μM E64d was used since a higher concentration of E64d (100 μM) was toxic to cells. Cell lysates were then analyzed for reactivity with anti-β3C by Western blot. As shown in Fig. 3, E64d significantly inhibited the loss of the β3 cytoplasmic domain immunoreactivity during apoptosis, indicating that calpain or a calpain-like protease is responsible for the cleavage of the β3 cytoplasmic domain. Similar to E64d, another membrane permeable calpain inhibitor, calpain inhibitor I, also inhibited cleavage of the β3 cytoplasmic domain (not shown).

Apoptosis Signaling—To determine whether the cleavage of β3 cytoplasmic domain was a consequence of apoptosis signaling, we tested the effect of a tyrosine phosphatase inhibitor, Na3VO4. We have shown previously that Na3VO4 blocks apoptosis of suspension-cultured HUVECs (17). Cells were incubated in suspension for 12 h in the absence or presence of Na3VO4. In agreement with previous results, Na3VO4 prevented apoptosis of suspension cultured endothelial cells as indicated by TUNEL assay (Fig. 5A). When these suspension-cultured cells were solubilized and immunoblotted with anti-β3 antibodies, we found that the β3 cytoplasmic domain was protected from cleavage in the presence of Na3VO4 (Fig. 5B). This effect was dose-dependent (data not shown) and correlated

**Fig. 3.** Calpain inhibitor E64d inhibits cleavage of the cytoplasmic domain of the integrin β3 subunit. HUVECs were cultured in suspension for increasing lengths of time in the absence (−E64d) or presence of 20 μM E64d (+E64d). Cells were then solubilized and equivalent amounts of lysates were analyzed by Western blots with anti-β3C (γC). Optical density was quantitated by scanning and analysis with NIH Image. Relative amounts in loading were estimated by Western blot with anti-β3. Relative reactivities are expressed as a percentage of the antibody reaction at the 0 time point (corrected by the loading factor).

**Fig. 4.** Cleavage of fodrin by calpain during suspension-induced endothelial cell apoptosis. HUVECs were cultured in suspension in the absence (No E64d) or presence of 20 μM E64d (+E64d) for increasing lengths of time, and then solubilized. Equivalent amounts of cell lysates were analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting with an antibody specifically recognizing a calpain cleavage site (GMMPR) in the N-terminal domain of the calpain-generated 150-kDa fodrin fragment but not intact fodrin.

**Fig. 5.** Effect of sodium vanadate on the suspension-culture induced loss of the cytoplasmic domain of the integrin β3 subunit. HUVECs were cultured either attached (Control), or in suspension for 12 h in the absence (No VO4), or presence of 100 μM sodium vanadate (100 μM VO4). A, cells were analyzed by TUNEL assay to indicate DNA fragmentation during apoptosis. B, cells were solubilized and immunoblotted with anti-β3C, directed against the cytoplasmic domain of β3 (relative amounts in loading were estimated by Western blot with anti-β3C). Note that inhibition of the loss of anti-β3C epitope in the presence of sodium vanadate correlates with inhibition of DNA fragmentation. This figure shows the results from three separate experiments (mean ± S.D.).
with the ability of Na₃VO₄ to block apoptosis (Fig. 5A). Thus, suspension culture per se is not responsible for the cleavage of β₃ cytoplasmic domain. Rather, cleavage of β₃ cytoplasmic domain occurs downstream of protein dephosphorylation in the apoptosis signaling pathway.

Cleavage of Integrin β₃ Cytoplasmic Domain during Apoptosis Induced by Serum Withdrawal—To investigate whether cleavage of the integrin cytoplasmic domain is unique in suspension-culture induced apoptosis, endothelial cells were induced to undergo apoptosis by serum withdrawal. As shown in Fig. 6, cells in serum-free medium became apoptotic, but at a much slower rate compared with that induced by suspension culture. Fig. 6 (D and E) shows a time-dependent reduction in the reaction of anti-β₃C with the integrin from endothelial cells cultured under serum-free conditions, indicating that cleavage of the integrin cytoplasmic domain occurred during apoptosis induced by serum withdrawal. Thus, cleavage of the integrin β₃ cytoplasmic domain is not unique for suspension-induced apoptosis, but is likely to be in the convergent signaling pathway induced by different apoptotic stimuli.

Cleavage of the β₃ Cytoplasmic Domain Is an Early Event, but Not a Consequence of Cell Death—We examined whether β₃ modification occurs as a step in the apoptosis signaling pathway or is a result of post-death proteolysis. To address this issue, we induced endothelial cell apoptosis by incubating cells in suspension culture for 12 h, then separated the late-phase apoptotic cells from cells in earlier phases of the apoptosis pathway. Cells were separated by their ability to re-adhere to serum-coated dishes (vitronectin is known to be the integrin ligand responsible for cell adhesion to serum-coated plates; Ref. 35). We found that nonadherent cells displayed the characteristic apoptotic morphology (not shown, cf. Fig. 1) while reattached cells appeared normal (Fig. 7). Lysates generated from both re-attached cells and nonadherent cells were then analyzed for immunoreactivity with the anti-β₃C antibody. Equivalent amounts of lysates from adherent endothelial cells were also examined as controls. In comparison with control cells, suspension-cultured cells showed significantly less (about 60%) reactivity with the anti-β₃C antibody even though these cells reattached to the matrix (Fig. 7). This indicates that cleavage of the β₃ cytoplasmic domain was initiated before cells lost the capacity to reattach. As dead cells do not adhere, this result suggests that integrin cleavage is not the result of post-death proteolysis. Furthermore, the reattached apoptotic cells appeared to have a normal morphology (Fig. 7, A and B), which also suggests that cleavage of β₃ is an early event and may be associated with the early phases of apoptosis signaling. Integrin β₃ from non-adherent cells showed further reduction in immunoreactivity with anti-β₃C compared with the cells reattached to serum-coated plates (Fig. 7, C and D). Similar results
were obtained when cells were replated on collagen or fibronectin (Fig. 7E). Thus, it appears that the majority of integrin β3 subunits are cleaved in late phase apoptotic cells that have lost their capacity to reattach and spread onto matrix proteins.

**Inhibition of the Loss of Cell Re-attachment Capacity by Calpain Inhibitor E64d**—The above study suggests a correlation between the loss of cell reattachment capacity and cleavage of the integrin cytoplasmic domain by calpain. To investigate whether calpain is indeed involved in regulation of cell adhesion during apoptosis, we examined the effects of E64d on cell readhesion. HUVECs were incubated in suspension for increasing lengths of time in the absence (−E64d) or presence of 20 μM (tE64d), and then allowed to reattach to tissue culture plates. Reattached cells were quantitated by cell counts, and results expressed as a percentage of total cells. A typical result of three experiments is shown. Paired Student’s t test of data from three experiments indicates that the inhibition by E64d at 6- and 12-h points is significant (p < 0.01).

**DISCUSSION**

In this study, we have shown that the cytoplasmic domain of integrin β3 subunit is cleaved during apoptosis in HUVECs (Fig. 2). Cleavage was detected by loss of reactivity with an anti-peptide antibody, anti-β3C, which recognizes the β3 C-terminal domain. We showed previously that anti-β3C immunoreactivity was lost when the cytoplasmic domain of β3 was cleaved by calpain (14). In apoptotic endothelial cells, the loss of anti-β3C immunoreactivity was inhibited by two different calpain inhibitors, E64d and calpain inhibitor I (Fig. 3). As these calpain inhibitors do not inhibit caspases (4), caspases are unlikely to be responsible. Also, as these calpain inhibitors do not inhibit DNA fragmentation of suspension-cultured endothelial cells (data not shown), it is unlikely that their effect was due to inhibition of suspension culture-induced apoptosis. Furthermore, cleavage of fodrin by calpain was also detected in the suspension cultured endothelial cells, suggesting that calpain is activated under these conditions (Fig. 4). Thus, the loss of reactivity with anti-β3C is likely to result from proteolytic cleavage of β3 by calpain. However, since we showed only a partial inhibition of integrin and fodrin cleavage by E64d, we cannot exclude the possibility that a different protease may also be involved.

Cleavage of the integrin β3 cytoplasmic domain is linked to the early phase of the convergent apoptosis signaling pathway. This conclusion is supported by several lines of evidence. First, cleavage of β3 was induced by two different apoptotic stimuli and was accompanied by induction of the apoptotic phenotypes (DNA fragmentation and morphological changes), suggesting that cleavage may be associated with the convergent apoptotic pathway. Second, cleavage of integrin was blocked by the phos-
phatase inhibitor sodium orthovanadate, an inhibitor that also blocks endothelial cell apoptosis induced by either suspension culture (Fig. 5) (17) or serum starvation (36), suggesting that the cleavage occurs downstream the activation of protein-tyrosine phosphatase activity in the apoptosis signaling pathway. Although it is not clear how protein phosphatases are involved, it is known that survival of anchorage-dependent cells requires signals initiated by adhesion receptors (e.g. integrins) and growth factor receptors, both of which activate protein-tyrosine kinases as early signaling mechanisms (for reviews, see Refs. 16 and 37). Either abrogation of integrin binding to matrix proteins or growth factor withdrawal may result in changes in the balance between the protein-tyrosine kinase and phosphatase activities, leading to dephosphorylation of intracellular proteins. Thus, the tyrosine phosphatase inhibitor is likely to function in the early phase of apoptosis pathway. Finally, the conclusion that calpain cleavage occurs in the early phase of apoptosis is supported by our finding that cleavage was initiated when cells were still able to readhere and showed apparent normal morphology (Fig. 7).

Cleavage of the integrin β3 cytoplasmic domain may negatively regulate integrin functions. Calpain cleavage sites in the β3 cytoplasmic domain have been identified in vitro and in platelets; these sites flank the two NXXY motifs (14). The NXXY motif is highly conserved among different integrin β subunits, including β1 and β2, which are also found in endothelial cells. We have evidence that the β3 cytoplasmic domain is also a calpain substrate. The NXXY motifs in β1 and β3 are required for the localization of integrins to focal adhesion sites, integrin-mediated tyrosine phosphorylation of signaling molecules such as focal adhesion kinase and also required for regulating ligand binding affinity (26, 28, 38). Truncation of the β3 cytoplasmic domain that removes C-terminal sequences containing the NXXY motif abolishes formation of the integrin-focal adhesion complex, abrogates integrin-mediated cell spreading, and inhibits integrin-mediated tyrosine phosphorylation (27, 29, 39, 40). Thus, it is possible that the cleavage of integrin β subunits, in concert with the cleavage of other focal adhesion proteins, leads to disruption of integrin-mediated adhesion, signaling, and cytoskeleton organization. In support of this viewpoint, we have observed a correlation between the extent of integrin cleavage and the ability of apoptotic cells to reattach (Fig. 7). In addition, we found that calpain inhibitor E64d significantly retarded the loss of cell reattachment (Fig. 8). Integrin-mediated signaling activates intracellular protein-tyrosine kinase and mitogen-activated protein kinase pathways, which are required for proliferation and survival of anchorage-dependent cells (41–45). Abrogation of integrin signaling induces apoptosis in endothelial cells and other anchorage-dependent cells (19–21). Thus, it is also possible that intracellular disruption of integrin survival signals by calpain cleavage, if occurring to adhere anchorage-dependent cells, serves as a feedback signaling mechanism that induces or accelerates apoptosis.

Interestingly, we found that the suspension cultured cells will re-adhere and spread when ~40% of β3 is cleaved, but lose the capacity to re-adhere when ~70% of the integrin is cleaved (Fig. 7). This is very similar to results from studies of patients with Glanzmann’s thrombasthenia, a genetic deficiency in the integrin αIIbβ3. Platelets obtained from heterozygotes (50% αIIbβ3 expression) showed no significant deficiency in adhesion while platelets expressing less than 30% of functional integrin were abnormal (46). Thus, it appears that cell reattachment may require only a fraction of the total integrins expressed.

However, the finding that cells with ~40% of integrin cleaved are still able to reattach does not suggest that the cleavage of a small percentage of integrin will not affect integrin function. Rather, it is possible that cleavage of a small population of integrins, if localized to existing integrin-focal adhesion sites of adherent cells, would abolish integrin signaling and cytoskeleton association in those specific focal adhesion sites, and thus promote cell rounding and detachment. Cleavage of integrin in existing focal adhesion sites of adherent cells is likely since calpain is colocalized in focal adhesion sites (47), and cleaves several focal adhesion proteins (9, 10, 13).

One approach to further determine the roles of calpain cleavage of integrins during apoptosis is to inhibit calpain cleavage of integrin. Experiments using calpain inhibitors, however, are complicated by the fact that calpain cleaves several intracellular proteins and plays multiple roles in cell survival and growth. Calpain inhibitors inhibit the cleavage and clearance of p53, which is pro-apoptotic, and thus induce apoptosis and growth arrest (48, 49). We also have found that high levels of E64d and calpain inhibitor I are toxic. In addition, calpain inhibitors may have nonspecific effects such as inhibition of proteasome activity by calpain inhibitor I (50). Multiple roles of calpain in cells explain previous contradictory results that calpain inhibitors both inhibit and induce apoptosis in different experiments (5–7, 51–54). Thus, further elucidation of the roles of cleavage of integrin in the apoptosis pathway awaits the development of new tools to specifically inhibit integrin cleavage without affecting the other cellular roles of calpain.

Acknowledgments—We thank Dr. Mark H. Ginsberg and Dr. Martin Schwartz for helpful discussions.

REFERENCES

1. Steller, H. (1995) Science 267, 1445–1449
2. Thompson, C. B. (1995) Science 267, 1456–1462
3. Martin, S. J., and Green, D. R. (1995) Cell 82, 349–352
4. Nicholson, D. W. A., A. T. N. Vaillancourt, J. P. Ding, C. K., Gallant, M., Greau, Y., Griffin, P. R., Labelle, M., Lazebnik, A., Y. Manda, N. A., Raju, S. M., Smulson, M. E., Yamin, T.-T., Yu, V. L., and Miller, D. K. (1995) Nature 376, 37–43
5. Squier, M. K., Miller, A. C., Malkinson, A. M., and Cohen, J. J. (1994) J. Cell. Physiol. 159, 229–237
6. Sarin, A., Adams, D. H., and Henkart, P. A. (1993) J. Exp. Med. 178, 1683–1700
7. Sarin, A., Clerici, M., Blatt, S. P., Hendrix, C. W., Shearer, G. M., and Henkart, P. A. (1994) J. Immunol. 153, 862–872
8. Martin, S. J., O’Brien, G. A., Nickolls, W. K., McAlton, A. J., Mabnobi, S., Saido, T. C., and Green, D. R. (1995) J. Biol. Chem. 270, 6425–6428
9. Cooyar, P., Yuan, Y., Schoenwaelder, S. M., Mitchell, C. A., Salem, H. H., and Jackson, S. P. (1996) Biochem. J. 318, 41–47
10. Oda, A., Druker, B. J., Ayyiush, H., Smith, M., and Salzman, E. W. (1993) J. Biol. Chem. 268, 12603–12608
11. Fox, J. E. (1994) Ann. N. Y. Acad. Sci. 714, 75–87
12. Saido, T. C., Yokota, M., Naga, S., Yamaura, I., Tani, E., Tsuchiya, T., Suzuki, K., and Kawanishi, S. (1993) J. Biol. Chem. 268, 25239–25243
13. Fox, J. E., Doll, G. D., Reynolds, C. C., and Phillips, D. R. (1995) J. Biol. Chem. 269, 1060–1066
14. Du, X., Saido, T. C., Toubuki, S., Indig, F. E., Williams, M. J., and Ginsberg, M. H. (1995) J. Biol. Chem. 270, 26146–26151
15. Hynes, R. O. (1992) Cell 70, 11–25
16. Schwartz, M. A., Schaller, M. D., and Ginsberg, M. H. (1995) Ann. Rev. Cell Dev. Biol. 11, 549–599
17. Meredith, J., and Schwartz, M. A. (1995) Mol. Biol. Cell 4, 953–961
18. Re, F., Zenati, A., Sironi, M., Polentarutti, N., Lanfrancon, L., Dejana, E., and Colotta, F. (1994) J. Cell. Biol. 127, 537–546
19. Brooks, P. C., Montgomery, A. M., Rosenfeld, M., Reisfeld, R. A., Hu, T., Klier, G., and Chereesh, D. A. (1994) Cell 79, 1157–1164
20. Meredith, J., and Schwartz, M. A. (1997) Trends Cell Biol. 7, 146–150
21. Frisch, S. M., and Francis, H. (1994) J. Cell Biol. 124, 619–626
22. Ruoslahti, E., and Reed, J. C. (1994) Science 267, 477–478
23. Chen, Y. P., O’Toole, T. E., Yianne, J., Rosa, P. J., and Ginsberg, M. H. (1994) Blood 84, 1857–1865
24. Chen, Y. P., O’Toole, T. E., Shipley, T., Forsyth, J., LaFlamme, S. E., Yamada, K. M., Shattil, S. J., and Ginsberg, M. H. (1994) J. Biol. Chem. 269, 8553–8558
25. O’Toole, T. E., Yianne, J., and Culley, B. M. (1995) J. Biol. Chem. 270, 8553–8558
Cleavage of Integrin during Apoptosis

27. Ylanne, J., Huuskonen, J., O'Toole, T. E., Ginsberg, M. H., Virtanen, I., and Gahmberg, C. G. (1995) J. Biol. Chem. 270, 9550–9557
28. Reszka, A. A., Hayashi, Y., and Horwitz, A. F. (1992) J. Cell Biol. 117, 1221–1330
29. Ylanne, J., Chen, Y., O'Toole, T. E., Lois, J., Takada, Y., and Ginsberg, M. H. (1993) J. Cell Biol. 122, 223–233
30. Akiyama, S. K., Yamada, S. S., Yamada, K. M., and LaFlamme, S. E. (1994) J. Biol. Chem. 269, 15961–15964
31. Chen, Y. P., Djaffar, I., Pidard, D., Steiner, B., Cieutat, A. M., Caen, J. P., and Rosa, J. P. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10169–10173
32. Hughes, P. E., Diaz-Gonzalez, G. F., Leong, L., Wu, C., McDonald, J. A., Shattil, S. J., and Ginsberg, M. M. (1996) J. Cell Biol. 130, 441–450
33. Uerrich, A., and Schlessinger, J. (1990) Cell 61, 203–212
34. Filardo, E. J., Brooks, P. C., Deming, S. L., Damaky, C., and Cheresh, D. A. (1995) J. Cell Biol. 130, 441–450
35. Leong, L., Hughes, P., Schwartz, M. A., Ginsberg, M. H., and Shattil, S. J. (1995) J. Cell Sci. 108, 3817–3825
36. Tahiliani, P. D., Singh, L., Auer, K. L., and LaFlamme, S. E. (1997) J. Biol. Chem. 272, 7892–7898
37. Lipfert, L., Hainovich, B., Schaller, M. D., Cobb, B. S., Parsons, J. T., and Brugge, J. S. (1992) J. Cell Biol. 119, 905–912
38. Chen, Q., Lin, T. H., Der, C. J., and Juliano, R. L. (1994) J. Biol. Chem. 269, 26602–26605
39. Zhu, X., and Assoian, R. K. (1995) Mol. Biol. Cell 6, 273–282
40. Leong, L., Hughes, P., Schwartz, M. A., Ginsberg, M. H., and Shattil, S. J. (1995) J. Biol. Chem. 270, 9550–9557
41. Lipfert, L., Hainovich, B., Schaller, M. D., Cobb, B. S., Parsons, J. T., and Brugge, J. S. (1992) J. Cell Biol. 119, 905–912
42. Chen, Q., Lin, T. H., Der, C. J., and Juliano, R. L. (1994) J. Biol. Chem. 269, 26602–26605
43. Zhu, X., and Assoian, R. K. (1995) Mol. Biol. Cell 6, 273–282
44. Chen, Q., Lin, T. H., Der, C. J., and Juliano, R. L. (1994) J. Biol. Chem. 269, 26602–26605
45. Frisch, S. M., Vuori, K., Ruoslahti, E., and Chan, H. P. (1996) J. Cell Biol. 134, 793–799
46. Beckerle, M. C., Burridge, K., DeMartino, G. N., and Croall, D. E. (1987) Cell 51, 569–577
47. Mellgren, R. L., Lu, Q., Zhang, W., Lakkis, M., Shaw, E., and Mericle, M. T. (1996) J. Biol. Chem. 271, 15568–15574
48. Shindo, H., and Usui, K. (1997) Mol. Cell. Biol. 17, 460–468
49. Shimizu, K., and Tomita, M. (1997) Biochim. Biophys. Acta 1314, 1130–1137
50. Gressner, A. M., Lahme, B., and Roth, S. (1997) Biochim. Biophys. Acta 1314, 1130–1137
51. Lu, Q., and Mellgren, R. L. (1996) Arch. Biochem. Biophys. 334, 175–181
52. Maeda, S., Lin, K. H., Inagaki, H., and Saito, T. (1996) Biochem. Mol. Biol. Int. 39, 447–453