Matrix Metalloproteinase 1 Interacts with Neuronal Integrins and Stimulates Dephosphorylation of Akt*

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Several studies have demonstrated that matrix metalloproteinases (MMPs) are cytotoxic. The responsible mechanisms, however, are not well understood. MMPs may promote cytotoxicity through their ability to disrupt or degrade matrix proteins that support cell survival, and MMPs may also cleave substrates to generate molecules that stimulate cell death. In addition, MMPs may themselves act on cell surface receptors that affect cell survival. Among such receptors is the \( \alpha_5\beta_1 \) integrin, a complex that has previously been linked to leukocyte death. In the present study we show that human neurons express \( \alpha_5\beta_1 \) and that pro-MMP-1 interacts with this integrin complex. We also showed that stimulation of neuronal cultures with MMP-1 is associated with a rapid reduction in the phosphorylation of Akt, a kinase that can influence caspase activity and cell survival. Moreover, MMP-1-associated dephosphorylation of Akt is inhibited by a blocking antibody to the \( \alpha_2\beta_1 \) integrin, but not by batimastat, an inhibitor of MMP-1 enzymatic activity. Such dephosphorylation is also stimulated by a catalytic mutant of pro-MMP-1. Additional studies show that MMP-1 causes neuronal death, which is significantly diminished by both a general caspase inhibitor and anti-\( \alpha_5 \) but not by batimastat. Together, these results suggest that MMP-1 can stimulate dephosphorylation of Akt and neuronal death through a non-proteolytic mechanism that involves changes in integrin signaling.

The matrix metalloproteinases (MMPs) represent a family of endopeptidases named for their ability to degrade extracellular matrix components. These enzymes play a role in tissue remodeling associated with both development and disease (1–7). In the central nervous system MMPs are released by cells including activated astrocytes, microglia, and neurons (8, 9).

Previously, we and others have shown that MMPs can be toxic to neurons in vitro (10–13). Although such toxicity may follow from extracellular matrix destruction (14), it is also reasonable to consider the non-mutually exclusive possibility that other mechanisms are involved. One extracellular matrix-independent mechanism by which MMPs may function involves their ability to cleave non-matrix proteins and thereby generate potential cell surface receptor signaling ligands (15–19). Another mechanism by which MMPs could affect cell survival might include direct effects on cell surface receptors. For example, MMP-9 binds to CD44 (20), MMP-2 binds to integrin \( \alpha_4\beta_1 \) (21), and pro-MMP-1 binds to integrins \( \alpha_5\beta_1 \) and \( \alpha_6\beta_1 \) (22). Such binding interactions may facilitate activation of the pro-enzyme, localize enzyme activity, disrupt cell matrix interactions to promote cell motility, and/or mediate internalization of the protease. Cell surface receptor binding by an MMP may also alter intracellular signaling. It has been shown that the snake venom metalloproteinase jararhagin can signal via \( \alpha_5\beta_1 \) on fibroblasts in a manner that is insensitive to inhibition of proteinase activity (23). In addition, we have shown that MMP-1 can stimulate protein release from neurons and monocytes in a manner that is insensitive to inhibition of its enzymatic activity (24). These results fit into a growing literature suggesting that enzymes can stimulate biological effects in a manner that is independent of their catalytic activity (25–27).

Although protein release has been examined as an end point of proteinase-associated \( \alpha_5\beta_1 \) signaling, other potential end-points have not been investigated. Of interest to the question of cytotoxicity, \( \alpha_5\beta_1 \) signaling has been linked to G\( _i \) protein-dependent cell death (28), presumably via a seven transmembrane CD47-\( \alpha_6\beta_1 \) complex (29). In addition, stimulation of \( \alpha_5\beta_1 \) with bovine collagen has been linked to protein phosphatase activation and the dephosphorylation of Akt (30). Akt is a critical effector of cell survival with targets that include Bad, caspase-9, and GSX-3B (31, 32). Reduced Akt activity has also been implicated in apoptosis mediated by diverse agents (33).

In the present study we have investigated the possibility that MMP-1 stimulation of neuronal cultures leads to the dephosphorylation of Akt. We have also examined the possibility that MMP-1 is linked to caspase inhibitor-sensitive cell death, a potential consequence of Akt dephosphorylation (34, 35).

EXPERIMENTAL PROCEDURES

Cell Culture—Brain specimens were obtained from human fetuses of 12–14 weeks gestational age with consent from women undergoing elective termination of pregnancy and approval by the Johns Hopkins University Institutional Review Board. Neuronal cultures were prepared as described previously (36). Briefly, the meninges and blood vessels were removed, and the specimens were washed in Opti-MEM (Invitrogen) before mechanical dissociation by repeated trituration through a 20-gauge needle. Cells were then pelleted at \( 270 \times g \) for 10 min and subsequently suspended in Opti-MEM with 5% heat-inactivated fetal bovine serum, 0.2% N2 supplement (Invitrogen), and 1% antibiotic solution (10 units/ml penicillin G, 10 \( \mu \)g/ml streptomycin, and 25 \( \mu \)g/ml amphotericin B) and plated in tissue culture flasks. The cells were maintained in culture for at least one month before neurons

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¶ The abbreviations used are: MMP, matrix metalloproteinase; GSX-3B, glycosyn thase kinase 3B; Akt, protein kinase B; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay.
were released through gentle shaking and then plated onto flat-bottomed plates, in which they were maintained for 5 days before experiments were conducted. The purity of cultures was established by immunostaining for microtubule-associated protein-2 (Dako, Carpinteria, CA).

**Immunostaining**—Neuronal cultures were grown on glass coverslips and fixed in 4% paraformaldehyde for 10 min at room temperature. The cells were washed three times with PBS, 5 min each, then blocked for 1 h in a PBS solution containing 4% donkey serum (Sigma) and 0.3% Triton X-100. This was followed by 4°C overnight incubation with 1:200 dilution of the primary antibody (mouse anti-human α5β1 or rabbit anti-human α5, both from Chemicon) diluted in 3% donkey serum. Normal mouse IgG was used as a negative control for α5β1 staining, and secondary antibody alone was used as a negative control for both α5 and α5β1 staining. Cells were washed 3 times in PBS for 5 min each and incubated for 2 h at room temperature in secondary antibodies diluted 1:500 in PBS (Alexa 488-conjugated anti-mouse from the Jackson Laboratory or goat anti-rabbit from Molecular Probes). Cells were again washed three times in PBS before mounting onto slides with Mowiol (Calbiochem). Slides were then analyzed using confocal (α5β1) or fluorescent (α5) microscopy.

**MMPs and Inhibitors**—Purified MMP-1 was purchased from Oncogene Research Products (San Diego, CA) and Chemicon International (Temecula, CA). The proteinase was separated in aliquots and stored at −70 °C upon its arrival. MMP-1 from Oncogene Research Products was purchased from human rheumatoid synovial fibroblasts by affinity chromatography, ion exchange chromatography, and gel filtration, and MMP-1 from Chemicon was purified from transfected PA2HA2 cells and also by ion exchange and affinity chromatography. The inactive pro-MMP-1 mutant, in which Glu-200 was changed to Ala (pro-MMP-1(E200A)), was overproduced in and purified from Escherichia coli according to methods previously described for the wild type enzyme (37).

Except where indicated MMP-1 from Oncogene Research Products was used in the experiments shown. As determined by Western blot, MMP-1 preparations from Chemicon contained both pro- and activated MMP-1, whereas that from Oncogene typically contained a majority of the inactive form. MMP-1 from Chemicon was used in the experiments shown. As determined by Western blot, MMP-1 contained both pro- and active forms (Chemicon, AB8105), whereas that from Oncogene typically contained a majority of the inactive form. MMP-1 was used in the experiments shown. As determined by Western blot, the supematant was incubated at 4°C overnight with anti-MMP-1 that recognizes pro- and active forms (Chemicon, AB8105), an isotype matched control antibody (Chemicon, AB5320), anti-α5β1 (Chemicon, MAB19982), or anti-β1 (Chemicon MAB2252). The mix was next incubated for 2 h with proteinase G-Sepharose, and after 3 washes the precipitate was analyzed by Western blot using a primary antibody to pro- and active MMP-1 (R&D Systems MAB901), α5β1 (Chemicon 19982), α5 (Chemicon AB1936); or β1 (MAB2252) as indicated. All incubations (antibody and protein G) were performed on a rotary table at 4°C, and all spins were performed using a desktop Eppendorf centrifuge at 4°C for 5 min at maximum speed (14,000 rpm).

**ELISA**—ELISA for phospho- and total Akt was performed using kits (KH00111 and KH00101) from BIOSOURCE International (Camarillo, CA). The phospho-Akt specific ELISA detects Akt, which is phosphorylated on serine 473. Both ELISAs were performed according to the manufacturer’s instructions. Although equal sample volumes were added to wells, correction for differing protein concentrations was made before calculation of relative Akt.

**Determination of Neuronal Cell Death**—At the time of experimental treatments, the culture medium was replaced with Locke’s buffer containing 154 mM NaCl, 5.6 mM KCl, 2.3 mM CaCl2, 1 mM MgCl2, 3.6 mM NaHCO3, 5 mM glucose, and 5 mM Hepes (pH 7.2). Cell death in each case was monitored by trypan blue exclusion 15 h after treatment of cells as described previously (12, 36). Neuronal cell counts were determined from five fields at predetermined coordinate locations. Each field was photographed and coded, and both live and dead cells were subsequently counted. A minimum of 100 cells was typically counted in each field. Each experiment was conducted in triplicate wells so that 15 fields were counted. Results were normalized so that control means were equal to 1. In these experiments MMP-1 was used at 50 ng/ml, ZVAD at 100 μM, pertussis toxin at 100 ng/ml, and batimastat at 20 ng/ml and 500 ng/ml (~1 μM). ZVAD and pertussis toxin were added in advance of MMP-1 (2 h for pertussis toxin and 30 min for ZVAD). For batimastat studies, MMP-1 was added to medium containing the inhibitor 30 min prior to the culture. Measurements were conducted on human neurons, we performed immunocytochemistry and Western blot analysis of lysates from these cells. As shown in Fig. 1, a–d, neurons showed immunoreactivity for α5β1 and α2. Staining was prominent along the cell body and was also present along cell processes. No staining was observed when normal mouse IgG or secondary antibody alone were used (not shown). In addition, expression of both α2 and β1 was confirmed by Western blot analysis of cell lysates (Fig. 1, d and e).

**RESULTS**

**Human Neurons Express α5β1**—Previous studies show that pro-MMP-1 binds to the I domain of the α5 integrin on keratinocytes (22). To determine whether this integrin is expressed on human neurons, we performed immunocytochemistry and Western blot analysis of lysates from these cells. As shown in Fig. 1, a–d, neurons showed immunoreactivity for α5β1 and α2. Staining was prominent along the cell body and was also present along cell processes. No staining was observed when normal mouse IgG or secondary antibody alone were used (not shown). In addition, expression of both α2 and β1 was confirmed by Western blot analysis of cell lysates (Fig. 1, d and e).

**MMP-1 Co-immunoprecipitates with Neuronal α5β1**—To determine whether MMP-1 associates with neuronal integrins, as it does with those expressed on keratinocytes (22, 38), we examined immunoprecipitates made using an antibody to α5β1 and to β1 for the presence of MMP-1. Results are shown in Fig.
**Fig. 1. Human neurons express** $\alpha_2\beta_1$. Immunostaining of human neurons for $\alpha_2\beta_1$ suggests that this integrin is expressed along the cell body (a) and also along processes (b). Staining of neurons for $\alpha_2$ shows similar expression (c) in an image at 100x. Contrast imaging of $\alpha_2$-stained cells is shown for comparison (d). Western blot analysis of two separately prepared neuronal extracts also shows a band at 165 kDa that is immunoreactive for $\alpha_2$ (e) as well as a band at $\sim$130 kDa that is immunoreactive for $\beta_1$ (f).

**Fig. 2. MMP-1 co-immunoprecipitates with $\alpha_2\beta_1$.** For the data shown in a immunoprecipitates were made using anti-$\alpha_2\beta_1$ or anti-$\beta_1$ as indicated at the top and analyzed by Western blot using a primary antibody to MMP-1. The lower panel shows immunoprecipitate lanes from an image obtained following a longer exposure of the membrane. 500 ng of purified MMP-1 was run in the control lane for comparison (shown in the upper panel only). The upper band represents the pro form and the lower band represents active length enzyme. For the data shown in b, immunoprecipitates were made using anti-MMP-1 and analyzed by Western blot using a primary antibody to $\alpha_2$ or $\beta_1$ as shown at the right. Neuronal lysates were run as controls. Immunoreactive proteins migrated according to their expected molecular masses (130 kDa for $\beta_1$ and 165 kDa for $\alpha_2$).

2a. The antibody used to make the precipitate is shown at the top, whereas that used to probe the blot is shown at the side. The upper panel is an image obtained after a relatively short exposure of film to membrane, whereas the lower panel is an image showing the immunoprecipitate lanes as they appeared after a longer exposure. In this experiment purified MMP-1 that had undergone some auto-activation was used as a control (shown in the upper panel only). Although the control was overloaded, two bands representing pro and active length MMP-1 can be appreciated. It can also be appreciated that, although cultures from which the precipitates had been made were treated with MMP-1 that contained near equal amounts...
MMP-1 and Akt

Fig. 3. MMP-1 stimulation of neurons leads to a decrease in intracellular phospho-Akt, and this is inhibited by a blocking antibody to \( \alpha_2 \). Human neurons were stimulated for 45 min with medium alone or medium containing 50 or 100 ng/ml MMP-1 or pre-treated for 30 min with anti-\( \alpha_2 \) (40 \( \mu \)g/ml) and then stimulated with 50 ng/ml MMP-1. Extracts were then prepared and compared for phospho-Akt. Decreased phospho-Akt in extracts from MMP-1-treated cells was observed with ELISA, which allowed for the quantification of Akt in numerous samples. Data shown represent the mean plus S.E. for phospho-Akt, calculated in units/mg of protein and then expressed as percent control in 5–5 replicates. In a the difference between unstimulated and MMP-1-stimulated extracts is significant at \( p < 0.03 \) (Mann-Whitney test). Total Akt was also measured by ELISA and was not decreased in association with MMP-1 (data not shown), implying that treatment was associated with dephosphorylation of Akt rather than degradation.

of both pro and active length forms and the blot had been probed with anti-MMP-1 that recognizes both forms, only pro length MMP-1 was detected. It is of course possible that active length MMP-1 can also associate with \( \alpha_2 \beta_1 \), but in our experiments its ability to do so was below the limits of detection. Of interest, the region of MMP-1, which is important to integrin binding, is not within the pro-domain but within the hinge and hemopexin domains, and yet it is also pro-MMP-1 that is detected in keratinocyte immunoprecipitates (22, 38). As shown in Fig. 2b, immunoprecipitates that were made using anti-MMP-1 also contained both \( \beta_1 \) and \( \alpha_2 \). Neuronal extracts were used as controls in this figure.

MMP-1 Stimulation of Neurons Is Associated with a Decrease in the Phosphorylation of Akt, and This Is Inhibited by a Blocking Antibody to \( \alpha_2 \)—We had previously shown that MMP-1 is toxic to human neurons, but the potential mechanisms were not investigated (12). Signaling through \( \alpha_2 \beta_1 \) in other cell types has been linked to protein phosphatase activation and subsequent Akt dephosphorylation (30). Reduced activity of Akt has in turn been linked to cell death (31). We, therefore, measured levels of phospho-Akt in untreated and MMP-1-treated neuronal cultures.

MMP-1 stimulation of human neurons was associated with reduced levels of phospho-Akt as determined by ELISA (Fig. 3a). Although MMP-1 from Oncogene was used in these experiments, similar results were obtained using a purified preparation from Chemicon. Also of note, the magnitude of the MMP-1-associated reduction in phospho-Akt was similar to that observed in fibroblasts after their stimulation with an established \( \alpha_2 \beta_1 \) agonist (30). To ensure that the reduction we observed in phospho-Akt was not due to degradation, total Akt levels were also measured by ELISA and were unchanged (data not shown). To examine the potential involvement of an \( \alpha_2 \) integrin-containing complex, we also tested MMP-1 on cultures that were pretreated with a blocking antibody to \( \alpha_2 \). Results demonstrate that anti-\( \alpha_2 \) attenuated the effect of MMP-1 (Fig. 3b). Because \( \alpha_2 \beta_1 \) has been linked to the activation of protein phosphatase 2A, we also tested the protein phosphatase inhibitor okadaic acid at a concentration relatively selective for protein phosphatase 2A for its effect on MMP-1-associated dephosphorylation of Akt. We observed that okadaic acid did reduce the ability of MMP-1 to reduce phospho-Akt while stimulating no significant increase on its own (50 ng/ml MMP-1, 74 ± 10% of control; 50 ng/ml MMP-1 plus 1 nm okadaic acid, 104 ± 8% of control, \( n = 3 \)).

The MMP-1-stimulated Reduction in Phospho-Akt Is Independent of Proteolytic Activity—Although immunoprecipitation studies suggest that MMP-1 may directly bind to \( \alpha_2 \beta_1 \), it is also possible that MMP-1 can generate a ligand that signals through \( \alpha_2 \beta_1 \) and/or other cell surface receptors to stimulate a decrease in phospho-Akt. Pro-MMP-1 undergoes autoactivation, and it is difficult to rule out activation of the pro-enzyme after its addition to cultured cells. We, therefore, tested batinstat, an inhibitor of the catalytic activity of MMP-1, for its ability to affect MMP-1-related changes in phospho-Akt. The results are shown in Fig. 4a and suggest that the ability of MMP to reduce phospho-Akt may be independent of proteolysis. As an additional experiment to assess the question of whether MMP-1 was acting in a manner independent of proteolysis, we tested the inactive pro-MMP-1 mutant in which Glu-200 was changed to Ala (pro-MMP-1 (E200A)). A glutamic acid residue at position 200 allows for interaction of a water molecule with the active site zinc, which in turn is critical for substrate catalysis (1, 39, 40). As shown in Fig. 4b pro-MMP-1 E200A also affected a statistically significant decrease in phosphorylated Akt.

MMP-9 Does Not Decrease Phospho-Akt—Like MMP-1, MMP-9 has been associated with neurotoxicity (10, 11). We, therefore, examined whether MMP-9 might also be associated with a reduction in phospho-Akt. In these experiments the active form of MMP-9 was used because it is this form of the enzyme that has been linked to cytotoxicity (11). Although MMP-1 was again associated with a significant reduction in phosphorylated Akt, levels in MMP-9-treated cells were similar to controls (Fig. 5). These data suggest that, at least for the cultures used and the time point examined in our studies the effect of MMP-1 is relatively specific.

MMP-1 Is Associated with Decreased Phosphorylation of GSK-3—\( \alpha_2 \beta_1 \) signaling has also been linked to decreased phosphorylation of GSK-3 (30), a potential effect of reduced serine-9 phosphorylation by Akt (41). We, therefore, examined lysates from unstimulated, MMP-1 stimulated, and pro-MMP-1 E200A-stimulated cells for phosphoserine 9 GSK-3. As shown in Fig. 6, MMP-1 and pro-MMP-1 E200A were associated with a reduction in serine 9-phosphorylated GSK-3,6 as determined by Western blot. Although additional studies will be necessary to better define the effect of MMP-1 on GSK-3, these data are consistent with effects previously reported for engagement of \( \alpha_2 \beta_1 \) by an alternate ligand (30) and, thus, lends
Further support to the idea that MMP-1 signals through this integrin.

**MMP-1-associated Neuronal Death Is Attenuated by ZVAD but Not by Batimastat—**Akt is thought to act as a pro-survival signal by inhibiting the activity of pro-apoptotic intracellular proteins including Bad. Overexpression of Akt will often promote cell survival and, perhaps more important with respect to the present study, stimuli that decrease Akt activity often induce apoptosis through a caspase-dependent mechanism (31).

As shown in Fig. 7 we tested the possibility that MMP-1 stimulation of neurons might lead to cell death in a manner that would be affected by ZVAD, an inhibitor of caspases. To determine whether MMP-1-induced neurotoxicity was dependent on its enzymatic activity, MMP-1 was also tested in the presence of batimastat. ZVAD was associated with a statistically significant inhibition of MMP-1-associated cell death (Fig. 7a). Batimastat, however, did not block cytotoxicity either at a relatively low dose (Fig. 7a) or at a high dose (1 μM, Fig. 7b).

Because of the differential responsiveness of primary cell cultures, there is some difference in MMP-1-associated toxicity between the two experiments (compare Fig. 7a to 7b).

As a further measure of toxicity, we also tested the ability of MMP-1 to affect mitochondrial membrane potential, which is reduced during early apoptosis (42). In these experiments the blocking antibody to αv was also tested for its ability to affect MMP-1-related changes. As shown in Fig. 7c, MMP-1 was associated with a reduction in mitochondrial membrane potential, as assessed by a fluorescent probe, and this change was not observed in cultures pretreated with the blocking antibody to αv. Along with the data shown in Fig. 7, a and b, these results suggest that MMP-1 is neurotoxic through a mechanism that is independent of proteolysis and involves changes in integrin signaling.

**DISCUSSION**

Although named for their ability to degrade matrix proteins and well studied for the same, MMPs are becoming increasingly recognized as effectors of cell signaling (43). For example, MMP-1 can degrade insulin-like growth factor-binding proteins and thereby stimulate IGF signaling (15), and MMP-9 may activate interleukin-1β (44). Although most studies have focused on the ability of MMPs to generate potential cell surface receptor binding ligands, MMPs can also bind to specific receptors, raising the possibility that they may more directly affect signaling. For example, MMP-9 can bind to CD44, MMP-1 can bind to integrins α5β1 and αvβ3, and MMP-2 can bind to integrin αvβ3 (20–22).

The present study shows that MMP-1 stimulates dephospho-
rylation of Akt through a mechanism that is attenuated by a blocking antibody to α2 but not by an inhibitor of enzymatic activity. These data suggest that MMP-1 may affect integrin signaling in a manner that is independent of proteolytic activity.

In terms of the receptor complexes and intracellular pathways linking MMP-1 to the dephosphorylation of Akt, it should be noted that integrin signaling has previously been linked to the activation of pathways that typically increase the phosphorylation of Akt. The engagement of α2β1 has, however, recently been reported to activate protein phosphatase 2A and to stimulate the dephosphorylation of Akt (30). Interestingly, a new study shows that Akt may be localized to protein phosphatase 2A-containing complexes, which may in turn be functionally linked to β1 (45). A specific tryptophan residue in the β1 cytoplasmic domain can specifically affect Akt signaling without having an effect on other integrin-activated pathways such as phosphoinositide 3-kinase (45). Of additional interest, integrin activation has also been linked to G protein signaling mediated by integrin-CD47 complexes (29). G protein signaling may also increase protein phosphatase activity (46, 47) and may, thus, lead to an overall decrease in the phosphorylation of Akt (30).

One possible consequence of MMP-1 signaling through α2β1 would be altered cell migration/process outgrowth. Integrins have been well studied for their effects on cell shape and migration (48). Of interest with respect to the possibility of metalloproteinase-integrin interactions and enhanced cell migration is a previous study showing that ADAM-9 interacts with α2β1 to stimulate fibroblast Rho kinase signaling and migration in a manner that is independent of catalytic activity (27). MMP-1-associated reductions in Akt activity could lead to changes in the activity of GSK-3β that would influence the stability of microtubule-associated proteins (41). Such changes might in turn have positive or negative effects on cell migration depending on factors including the type of cell and its status. It is, therefore, tempting to speculate that although MMPs disrupt matrix integrity and by doing so create extracellular conditions which allow for changes in cell shape and/or migration, they might also stimulate requisite intracellular changes.

Another potential consequence of altered signaling through α2β1 by MMP-1 is reduced cell survival. Apoptosis is required for tissue remodeling such as that which may occur in disease/inflammation. MMP-1 may engage unoccupied α2β1 and thereby stimulate intracellular changes linked to reduced cell survival. MMP-1 might also displace an endogenous ligand or otherwise alter the shape and/or adhesive properties of such a
ligand, thereby altering $\alpha_{i}\beta_{1}$ signaling and/or promoting weak adhesion. Although intermediate adhesion has been associated with cell motility, weak adhesion has been linked to cell death by anoikis (49). The cells used in our experiments were not plated on collagen or other known $\alpha_{i}\beta_{1}$-binding proteins, but we cannot rule out their production of potential ligands or cell-cell interactions leading to $\alpha_{i}\beta_{1}$ engagement.

In the present study neurotoxicity was inhibited by a blocking antibody to $\alpha_{i}$, suggesting that changes in integrin signaling played a role. Integrin signaling has more generally been linked to cell survival. The ability of changes in integrin signaling to stimulate survival or apoptosis is likely, however, to depend on the nature of both the integrin and the ligand. With respect to $\alpha_{i}\beta_{1}$, smooth muscle cell death has recently been reported to follow stimulation of cells with an $\alpha_{i}\beta_{1}$-activating monoclonal antibody (28), and T cell apoptosis has been linked to the activation of $\beta_{i}$ (50). The recently described functional link between $\beta_{i}$ and protein phosphatase 2A (45) may be relevant with respect to these observations as well as to our own. In the study describing this functional link it was proposed that some $\beta_{i}$ interactions are likely to maintain $\alpha_{i}$-associated Akt protein phosphatase 2A complexes in a state so that protein phosphatase 2A activity is repressed (45). Conceivably, effects on $\beta_{i}$ by specific ligands or by loss of matrix might derepress protein phosphatase 2A and, thus, lead to decreased activity of Akt and apoptosis.

Our findings also suggest that MMP-1-associated toxicity may be caspase-dependent. Although dephosphorylation of Akt would be expected to promote caspase-dependent apoptotic death, additional studies will be necessary to determine the extent to which MMP-1-related death may depend on changes in the activity of Akt as well as on changes in the activity of specific caspases.

In summary, we have shown that MMP-1 stimulation of neurons leads to Akt dephosphorylation and cell death through a mechanism that is independent of proteolysis. These findings suggest that MMP-1 might contribute to the neuronal damage which occurs in association with degenerative and inflammatory conditions characterized by elevated levels of this proteinase.

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