PR-39, a Syndecan-inducing Antimicrobial Peptide, Binds and Affects p130Cas§

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PR-39 is a proline-arginine-rich antimicrobial peptide and an important component of innate immunity. In addition to its antimicrobial effects, PR-39 can alter mammalian cell gene expression and behavior. To determine the mechanism through which PR-39 affects mesenchymal cells, we identify a number of binding targets for PR-39 using a biologically active fragment of PR-39 (PR-39(15)). We found that PR-39 binds NIH 3T3 in a saturable manner consistent with the existence of a binding target. Similar to full-length PR-39, PR-39(15) interacts with lipid bilayers. After interacting with the membrane, PR-39(15) rapidly enters human microvascular endothelial cells and binds a number of cytoplasmic proteins. PR-39 selectively binds recombinant SH3-containing proteins and was also found to bind a native SH3-containing protein, p130Cas. PR-39(15) treatment of endothelial cells results in altered p130 localization. These results show that PR-39(15) binds an SH3-containing signal transduction molecule that has the potential to explain a myriad of effects PR-39 has on mammalian cell behaviors.

Antimicrobial peptides are important elements of innate host defense in a wide variety of organisms. They are expressed at biological boundaries susceptible to infection, and their rapid production and diffusibility put them in a unique position for early protection against microbial invasion (1). Antimicrobial peptides are attracting increasing interest in light of the developing threat of antibiotic-resistant pathogens. Furthermore, recent developments have shown that these peptides are expressed in response to a number of inflammatory events such as wound repair (2–4). These peptides may modulate host cellular responses by affecting behaviors like syndecan expression (5), chemotaxis (6), and chloride secretion (7). Thus, individual peptides in this diverse set of molecules have exhibited diverse functions. However, the exact mechanisms of action of many of these peptides have yet to be elucidated.

A recently identified class of antimicrobial peptides, the cathelicidins, has been the focus of our studies. Precursors of cathelicidins, has been identified in pig gut and neutrophil azurophilic granules (11–13). PR-39 is active against several strains of Gram-positive and Gram-negative bacteria (14). In contrast to a classical pore-forming killing mechanism established for many other antimicrobial peptides, it was found that PR-39 halts protein and DNA synthesis (15). Despite lack of evidence for membrane permeabilization, PR-39 does demonstrate membrane activity (16). This behavior presents an intriguing potential mechanism for PR-39’s numerous effects on mammalian cells. For example, PR-39 is a neutrophil chemoattractant (6). PR-39 also induces syndecan expression on mesenchymal cells (5). Syndecans are proteoglycans known to play a role in wound repair (17, 18). As a result of increased syndecan expression, PR-39 has also been found to influence cell motility and metastatic potential (19). Thus, PR-39 is a multifunctional peptide that can affect both microbial and host cellular processes via mechanisms that are yet to be determined.

To explore the mechanism by which PR-39 may affect mesenchymal cells, we identified proteins that bound PR-39. We establish that PR-39 binds NIH 3T3 in a saturable manner, consistent with the existence of a specific binding target. In our studies, we used a 15-amino acid N-terminal fragment of PR-39 (PR-39(15)) as a probe for potential binding targets. We found that PR-39(15) interacts with the membrane, enters cells, and binds intracellular SH3-containing proteins. Of these proteins, we chose to further characterize PR-39 binding to p130Cas, an adapter protein that has been implicated in numerous signaling pathways. Finally, we show that a consequence of PR-39(15) treatment of endothelial cells is altered p130 localization. This finding has the potential to explain many effects of PR-39 on intracellular signaling and resultant changes in gene expression or behavior.

EXPERIMENTAL PROCEDURES

Cell Culture—NIH 3T3 were grown in Dulbecco’s modified Eagle’s medium containing glucose at 4.5 g/liter, 10% defined bovine serum (HyClone, Logan, UT), 100 units/ml penicillin, and 100 μg/ml streptomycin. Human dermal microvascular endothelial cells were purified from foreskin using magnetic beads coated with anti-E selectin monoclonal antibody as described previously (20). Homogeneous cell populations were confirmed by morphology and expression of CD31 or von Willebrand factor. Cells were grown in EBM (Clonetics, Walkersville, MD) supplemented with 1 μg/ml hydrocortisone acetate, 5 × 10⁻⁵ M N6,2'-O-dibutyryl-adenosine 3',5'-cyclic monophosphate (Sigma), 20%
fetal calf serum (HyClone), 100 units/ml penicillin, and 100 µg/ml streptomycin. All experiments using endothelial cells were performed using cells at less than passage 7.

Peptides—All peptides used were synthetically made. Full-length PR-39 (Chiron Mimotopes Peptide Systems, San Diego, CA) was 96% pure by high pressure liquid chromatography. A fragment containing the first 15 residues of PR-39 (PR-39(15)) was synthesized and biotinylated on a cysteine residue added to the C-terminal end (QCB, Hopkinton, MA). Biotinylated PR-39(15) was 95% pure by high pressure liquid chromatography. A 12-mer peptide (PR-39(12)) missing the first three arginines from the N-terminal end was derived from PR-39(15). Synthetic PR-39(12) was 97% pure by high pressure liquid chromatography and was biotinylated as described above. Both peptide fragments were tested for antimicrobial activity by well diffusion assay as described previously (21).

Radioligand Binding and Analysis—The association of PR-39 with cells was evaluated with NIH 3T3 cells grown to confluence in 24-well tissue culture plates (Costar, Cambridge, MA). PR-39 was iodinated by IODO-GEN™ bead technique (Pierce) to a specific activity of approximately 5 × 10⁶ cpm/µg protein. For evaluation of binding of PR-39 to cells over time, 125I-PR-39 was added to wells in triplicate at a concentration of 0.37 µg/ml in NIH 3T3 cell culture media and to identical wells to which a 20-fold excess of unlabeled PR-39 was added simultaneously. Cells were incubated at 37 °C for various times, then gently washed 3 times with ice-cold PBS (140 mM NaCl, 5.0 mM KCl, 0.05 mM NaH₂PO₄, 2 mM KH₂PO₄, pH 7.4) or TBS (1 mM Tris-HCl, pH 7.4) containing either 150 mM NaCl or 15 mM KCl. After incubation the cell monolayer went through two cycles of PBS washes and was lysed with 0.5 ml PBS (140 mM NaCl, 3 mM KCl, 10 mM NaH₂PO₄, 2 mM KH₂PO₄, pH 7.4) or TBS (1 mM Tris-HCl, pH 7.4) containing 150 mM NaCl or 15 mM KCl. Cell lysates were then washed and solubilized in 0.1% SDS-RIPA for 30 min at 4 °C and supernatants were added to 100 µg protein. For cytoskeletal p130 analysis, endothelial cells were cultured as described above and incubated with 0–62.5 µg/ml PR-39(15) at 37 °C. Each concentration was tested in triplicate. Cells were washed with ice-cold PBS and lysed for 2 min at 4 °C in cytoskeleton stabilization buffer (CSK) (0.3 mM sucrose, 0.1% Triton X-100, 10 mM PIPES, pH 6.8, 100 mM KCl, 1 mM CaCl₂, 2.5 mM MgCl₂, 50 mM NaF, 0.1 mM Na₃VO₄). Insoluble cell fractions were solubilized in 0.1% SDS-RIPA for 30 min at 4 °C with vigorous agitation. Lysates were homogenized with a 27-gauge needle and run on a 10% SDS-PAGE. Immunoblot analysis with anti-p130 was then performed.

For cytotoxic p130 analysis, endothelial cells were cultured as described above and incubated with 0–62.5 µg PR-39(15) at 37 °C. Each concentration was tested in triplicate. Cells were washed with ice-cold PBS and lysed for 2 min at 4 °C in cytoskeleton stabilization buffer (CSK) (0.3 mM sucrose, 0.1% Triton X-100, 10 mM PIPES, pH 6.8, 100 mM KCl, 1 mM CaCl₂, 2.5 mM MgCl₂, 50 mM NaF, 0.1 mM Na₃VO₄). Insoluble cell fractions were solubilized in 0.1% SDS-RIPA for 30 min at 4 °C with vigorous agitation. Lysates were homogenized with a 27-gauge needle and run on a 10% SDS-PAGE. Immunoblot analysis with anti-p130 was then performed.

RESULTS

PR-39 Binds Cells in a Receptor-dependent Manner—The interaction of PR-39 with cells was studied in order to determine if there was a receptor for PR-39. An association rate assay was done to determine the rate at which 125I-PR-39 binding to NIH 3T3 would reach equilibrium (Fig. 1A). The specific binding data were fit to the following one-phase exponential association equation: Y = Ymax(1 − e−kobs t), Y is 125I-PR-39 saturable binding in cpm × 10⁶; Ymax is the maximum binding achieved for the concentration of 125I-PR-39 used (same units as Yt); kobs is the observed rate constant in min⁻¹, and t is time in minutes (25). The data were fit to the model using nonlinear least squares regression in order to determine values for Ymax and Kdobs (Table I). The coefficient of determination (R²) is 0.9773, indicating that 97.7% of the variation in the data is explained by the equation and therefore that the system behaves in accordance with the model. From the curve fit to the data, we determined that binding reaches equilibrium after 60 min. This information was then used in an assay to determine concentration dependence of PR-39 binding (Fig. 1B).

Various concentrations of 125I-PR-39 were incubated with confluent NIH 3T3 for 60 min. The binding data were fit using the same technique as above to the following equation: B = (Bmax[I]/(Kd + [I]) + Bobs). B is the specific binding in nanomoles/well; Bmax is the total number of receptors expressed in the same units as B; [I] is the concentration of free radioligand in µM, and Kd is the equilibrium dissociation constant expressed.
in the same units as [L]. $B_{\text{max}}$ and $K_d$ were the coefficients estimated (see Table I, part B). $R^2$ is 0.9888 indicating that the data fit the model well. A two-binding site model was fit to the data to test the possibility that there were two populations of receptors. The second $K_d$ estimated in this process was nonsensical (negative value) so that model was rejected. The results indicate that $^{125}$I-PR-39 binds NIH 3T3 in a saturable manner and with a $K_d$ of 0.03 m.

A biotinylated 12-amino acid fragment derived from PR-39 (15) that is missing the first three arginine residues (PR-39(15)) also retained the membrane activity shown for full-length PR-39. Treatment of NIH 3T3 pretreated with 0.2% Triton stain brightly with propidium iodide (Fig. 3C, whereas NIH 3T3 pretreated with 0.2% Triton stain brightly with propidium iodide (Fig. 3D).

PR-39 Binds Multiple Cytosolic Proteins—Since PR-39 interacts with membrane, enters cells, and localizes to the cytoplasm, we studied cytosolic proteins for potential binding targets. NIH 3T3 were metabolically labeled with [35S]methionine/[35S]cysteine, and the cytosolic fraction containing SH3—containing proteins, as predicted by its proline-rich sequence was obtained. This fraction was incubated with immobilized PR-39(15), and bound proteins were eluted with an increasing NaCl gradient. Total counts/min for each elution was measured (Fig. 4A). Fractions collected were also evaluated by SDS-PAGE (Fig. 4B). The flow-through fraction (0.15 M NaCl) shows homogeneous labeling of cytosolic proteins. Many PR-39(15) binding proteins eluted at 0.4 M NaCl. However, a limited set of distinct protein bands binding to PR-39(15) was observed in the 1.6 M NaCl eluate. This NaCl fraction corresponded with a peak in radioactive counts observed in Fig. 4A.

PR-39 Selectively Binds Recombinant Proteins Containing SH3—To define further candidate binding proteins for PR-39, we used a gel-shift assay to confirm that PR-39 bound SH3-containing proteins, as predicted by its proline-rich sequence and as previously reported (26). Recombinant proteins containing SH3 domains bound $^{125}$I-PR-39 and produced a band that shifted up in molecular weight, whereas non-SH3-containing proteins did not change the signal from baseline $^{125}$I-PR-39 alone (Fig. 5).

PR-39 Binds $p^{130^\text{Cas}}$—Guided by location of bound proteins and molecular weights of eluted proteins from the assay in Fig. 4, we evaluated SH3-containing proteins as candidate-binding targets. We identified $p^{130^\text{Cas}}$, a native SH3-containing protein, as a PR-39(15)-binding protein by immunoprecipitation. Endothelial lysates were immunoprecipitated with anti-p130 (Fig. 6A) or anti-PR-39 (Fig. 6B) in the presence of PR-
PR-39(15) or biologically inactive PR-39(12) as control. Potential PR-39(15)-p130 complexes were evaluated by immunoblotting with anti-PR-39 or anti-p130 as indicated. A dot blot done previously determined that anti-PR-39 detects both PR-39(15) and PR-39(12) (data not shown). PR-39(15) was detected in complexes immunoprecipitated with anti-p130 but PR-39(12) was not. In the reverse experiment, p130 was detected in anti-PR-39 immunoprecipitates of endothelial lysates that were mixed with PR-39(15) but not in lysates mixed with PR-39(12). Thus, biologically active fragments of PR-39 bind p130 whereas similar but inactive peptides do not.

PR-39(15) Induces a Decrease in Cytosolic p130 and Increase in Cytoskeletal p130 in Endothelial Cells—To determine if PR-39 affects p130 subcellular distribution, we evaluated p130 localization in endothelial cells after PR-39(15) treatment. We evaluated cytosolic p130 levels in confluent endothelial cells treated with 10 μM PR-39(15) from 2 to 8 h. PR-39(15) treatment lowered the amount of p130 in the cytosolic fraction (Fig. 7A). Next, the amount of p130 in the cytoskeletal compartment was measured with respect to PR-39(15) concentration. The maximum increase in cytoskeletal p130 occurred after a 6-h treatment, increasing detectable p130 by 2–4-fold (p = 0.02). Thus, endothelial cells were treated with 0–62.5 μM PR-39(15) for 6 h and cytoskeletal p130 levels then measured (Fig. 7B). Dose-response data from three trials were fit to an equation of the form $Y_{id} = a + bx_{id} + cx_{id}^2 + \epsilon_{id}$ using ordinary least squares, where $Y$ is p130 response; $i$ is the trial number; $d$ is the dose number, and $\epsilon$ is an error term we assume distributed identically and independently across dose and trial. This was a reasonable assumption because all treatments were done at the same time on the same plate. Lysis conditions were identical, and all samples were loaded on the same gel. A quadratic equation was chosen because the data appeared to exhibit a dose-dependent increase in p130 with respect to PR-39(15) concentrations. However, the 62.5 μM PR-39(15) treatment produced diminished responses in p130 (data not shown). This diminished response is expected because PR-39 becomes toxic to eukaryotic cells at higher concentrations. The coefficients estimated are shown, and their $p$ values indicate that they are significantly different from zero (Table II). The first derivative of the equation is positive for lower concentrations of PR-39(15) indicating an increase in p130 with respect to PR-39(15) concentration. However, the negative sign on the second derivative indicates a decreasing marginal response of p130 as the PR-39(15) concentration increases. This statistical analysis confirms the observation that increasing PR-39(15) concentrations resulted in increased cytoskeletal p130 to complement the decrease in cytosolic p130 seen in Fig. 7A.

DISCUSSION

Antimicrobial peptides are rapidly produced, rapidly diffusible components of the innate immune system (27, 28). Their presence on epithelial surfaces provides a powerful early response to microbial infection. Recent work has demonstrated that antimicrobial peptides are multifunctional effectors whose actions extend beyond inhibiting microbial proliferation. These functions include regulation of cell proliferation, extracellular matrix production, and cellular immune responses (1). However, the exact mechanisms of action of these peptides are still unclear. We have studied the cathelicidins, a diverse group of peptides with wide spectrum bactericidal activity (8). We previously found that a proline-arginine-rich group of peptides within this family of antimicrobial peptides induced syndecan expression during wound repair (5). Additionally, it was found that these proline-arginine-rich peptides did not kill via a pore-forming mechanism, but by affecting protein and DNA synthesis (15, 29). Furthermore, the amphiphilic characteristics of these peptides present an interesting possibility that membrane interaction may play a role in how these peptides effect changes in gene expression. The work we present here further elucidates a mechanism of action of one of these proline-arginine-rich peptides, PR-39.
PR-39 is a unique antimicrobial peptide that was identified in pig gut and neutrophils (11–13, 30). It has been demonstrated that PR-39 induces syndecan expression in mesenchymal cells (5), inhibits neutrophil oxidase (26), and is a chemotactic agent for neutrophils (6). Because PR-39 has been shown to have all these behavior-modulating effects without extensive membrane permeabilization, we attempted to identify binding targets through which PR-39 would exert its effects. Our results in Fig. 1 demonstrate that PR-39 binds fibroblasts in a saturable manner, confirming the existence of a receptor or specific binding target for PR-39. However, this binding was inhibited at 4 °C, suggesting that membrane fluidity may affect binding. In our studies, we used a truncated peptide consisting of 15 N-terminal residues of PR-39 (PR-39(15)) that retains the biological activity of full-length PR-39. This approach led to the detection of multiple potential cytoplasmic binding targets. Among these a Crk-associated substrate (Cas), p130Cas, was of interest because of its role in integrin-mediated signaling pathways. We present evidence that a consequence of PR-39(15) treatment is altered p130 localization. Our work suggests that PR-39 can directly modify p130 signaling pathways.

We began by studying basic properties of PR-39(15) to establish that it would be an adequate probe for binding targets. It has been shown that a 26-amino acid N-terminal fragment retains antimicrobial activity (29). We established that an even shorter fragment, PR-39(15), retained its antimicrobial activity in a well diffusion assay. The temperature dependence of PR-39 binding as shown by saturation binding experiments suggested that PR-39 was not binding a cell-surface receptor. Also, recent work on protamine, another proline-arginine-rich antimicrobial peptide, demonstrated that it disrupted energy transduction and nutrient uptake, suggesting that the plasma membrane was the site of action (31). Thus, we explored the possibility that PR-39(15) could be targeting the plasma membrane by studying its behavior in a lipid bilayer system. The sequence of PR-39 is highly hydrophobic, and it has been found

![Image](image.png)
that full-length PR-39 partitions into lipid membranes (16). First, we found that the electrophysiological activity of PR-39(15) is similar to that of full-length PR-39. PR-39(15) perturbs lipid bilayers when a critical concentration of PR-39(15) is used, but the erratic behavior of PR-39(15) made it difficult to obtain an $I(V)$ curve or establish any further concentration dependence. Then, we show a new finding for PR-39(15); minute ion channels are formed (Fig. 2). However, the integrity of the membrane is breached shortly thereafter if the voltage potential is sustained, a result consistent with previous reports. It was suggested that PR-39 may have a polyproline type II structure that enables it to interact with cell membranes (16), and related peptides have been shown to form polyproline type II helices that disrupt membranes, forming channels (32, 33). These results show that PR-39(15) interacts with lipid bilayers similar to full-length PR-39 and may affect cellular processes by perturbing ion gradients.

In an effort to localize PR-39(15)-binding targets, we examined PR-39(15) cell staining patterns with fluorescence microscopy. Interestingly, we observed a cytoplasmic pattern of staining, suggesting that PR-39(15) also crosses the membrane after interacting with it (Fig. 3). The cytoplasmic staining was observed within 5 min, indicating that partitioning into the membrane and cell entry occurs rapidly. Based on this observation, we tried to purify the PR-39(15)-binding protein by examining cytosolic preparations from NIH 3T3. The results in Fig. 4 indicate that PR-39(15) strongly binds multiple cytosolic proteins, a number of which were high molecular weight. Although both of these results do not exclude the possibility that PR-39(15) effectors may also exist in the nucleus or plasma membrane, they reliably indicate that there are PR-39(15)-binding proteins in the cytoplasm.

The proline-rich sequence of PR-39 bears a number of SH3 binding motifs. Thus, a candidate-binding target containing SH3 domains would be consistent with the cytoplasmic localization of PR-39(15) as well as previous reports (26). An in vitro binding assay with SH3-containing proteins confirmed that PR-39 does preferentially bind the SH3 domain as predicted. Thus, we had cumulatively established that PR-39(15) binds a number of high molecular weight cytoplasmic targets that may contain an SH3 domain. Using this information, we tested binding of PR-39(15) to various native SH3 proteins by immunoblot. We found that PR-39(15) bound a number of native SH3 proteins. One binding protein we chose to characterize further, p130Cas, was a recently cloned adapter molecule that was identified as a v-Crk-associated substrate (Cas) with multiple phosphorylation states (34, 35). We chose to test p130 because it was known to interact with proline-rich regions of other signal transduction components via its SH3 domain (36–38). In addition, p130 is found in focal adhesions (37, 39), where integrins colocalize with syndecans (40), a proteoglycan that is induced by PR-39 during wound repair. The binding of p130 to other components of signal transduction is dependent on tyrosine phosphorylation of p130 (41), and subcellular fractionation shows that p130 could move to the membrane upon tyrosine phosphorylation (34). Furthermore, conditions known to result in an effort to localize PR-39(15)-binding targets, we examined PR-39(15) cell staining patterns with fluorescence microscopy. Interestingly, we observed a cytoplasmic pattern of staining, suggesting that PR-39(15) also crosses the membrane after interacting with it (Fig. 3). The cytoplasmic staining was observed within 5 min, indicating that partitioning into the membrane and cell entry occurs rapidly. Based on this observation, we tried to purify the PR-39(15)-binding protein by examining cytosolic preparations from NIH 3T3. The results in Fig. 4 indicate that PR-39(15) strongly binds multiple cytosolic proteins, a number of which were high molecular weight. Although both of these results do not exclude the possibility that PR-39(15) effectors may also exist in the nucleus or plasma membrane, they reliably indicate that there are PR-39(15)-binding proteins in the cytoplasm.

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in increased phosphotyrosine in p130 (integrin-mediated cell adhesion and Src transformation) corresponded with elevated complexes of p130-FAK in nonionic detergent-insoluble fractions, suggesting increased cytoskeletal association (24).

Therefore, we tested the effects of PR-39(15) treatment on p130 localization by subcellular fractionation. Our work shows that PR-39(15) treatment causes cytosolic p130 levels to drop, whereas cytoskeletal p130 exhibits a dose-dependent increase with respect to PR-39(15). This suggests enhanced p130 association with the cytoskeleton upon PR-39(15) treatment, a state which is consistent with a number of processes that lead to activation of p130-associated signaling pathways.

Diverse signaling pathways in many different cell types have now been linked to p130: integrin-signaling, G-protein coupled receptors (42–44), tyrosine kinase receptors (45), and mitogen-mediated signal transduction (46) have all implicated p130 in their signaling processes. In addition, p130 plays a role in cell transformation (34, 47–49) and is phosphorylated following KCl-induced depolarization by a Ca\(^{2+}\)-dependent mechanism (50). The integrin-mediated signaling pathway is interesting because of its potential to explain previous findings regarding the effect of PR-39 on mesenchymal cells. The colocalization of syndecans and integrins in focal adhesions could be further linked by convergent signal transduction processes that are centered around p130. PR-39 treatment results in syndecan induction in the focal adhesion, and this in turn could either lead to synergistic activation or negative feedback resulting in down-regulation of integrin-associated functions. Hepatoma cells transfected with PR-39 show increased expression of syndecan-1 and suppressed migration on fibronectin (19). Meanwhile, FAK promotes cell migration on fibronectin, and this

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**TABLE II**

| Coefficient estimates and standard errors of quadratic equation used to describe p130 dose-response behavior |
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| Dose-response data from Fig. 7B were fit to the equation in the first line of the table. Values for \(a\), \(b\), and \(c\) were estimated by linear regression, and \(p\) values for the estimates are shown. |
| Model: \(Y_{id} = a + bx_{id} + cx_{id}^2 + \epsilon_{id} \) |
| Coefficient | Estimate | Standard error | \(t\) | \(p\) value |
|---|---|---|---|---|
| \(a\) | 70.55599 | 9.021497 | 7.821 | 0.000 |
| \(b\) | 3.573681 | 1.10149 | 3.244 | 0.004 |
| \(c\) | -0.0549214 | 0.0175879 | -3.123 | 0.005 |
| \(R^2\) | 0.3339 | |

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**FIG. 6.** PR-39(15) binds p130Cas. A, 10 nmol of PR-39(15) alone, detected by immunoblot with anti-PR-39(15) (lane 1). PR-39(15) (lane 2) or PR-39(12) (lane 3) was added to dermal endothelial cell lysate. Lysate was immunoprecipitated (IP) with anti-p130, and complexes were immunoblotted with anti-PR-39(15). B, p130 detected in total dermal endothelial cell lysate by immunoblot with anti-p130 (lane 1). Peptides were added to lysates as in A. Lysate was immunoprecipitated with anti-PR-39(15), and complexes were immunoblotted with anti-p130.

**FIG. 7.** PR-39(15) causes p130Cas to decrease in cytosol and increase in cytoskeleton. A, PR-39(15) causes a decrease in cytosolic p130. Confluent dermal endothelial cells were incubated with and without 10 \(\mu\)M PR-39(15) at 37 °C for the indicated times, and cytosolic fractions were purified as described under “Experimental Procedures.” B, PR-39(15) causes dose-dependent increase of p130 in cytoskeletal fractions. Confluent dermal endothelial cells were incubated in various concentrations of PR-39(15) at 37 °C for 6 h. Cytosolic fraction was removed, and remaining cytoskeletal fraction was solubilized for immunoblot analysis of p130 as described under “Experimental Procedures.” Densitometric image analysis on the blot converted band intensity to the index plotted on the y axis. Results on graph reflect average response over three trials.
process requires p130 binding (51). These results point to PR-39 having a negative feedback effect on integrin function which is inconsistent with the findings presented here. However, concentration dependence may be a key modulator of the final response. For example, p130 phosphorylation exhibits a bell-shaped dose-response curve in response to epidermal growth factor stimulation (52). Similarly, we observed decreasing marginal increase of cytoskeletal p130 in response to rising PR-39(15) concentrations (Fig. 7B). This composite of evidence suggests that the effect PR-39 has on p130 may be more complex than simply binding and shifting p130 between supposed inactive and active pools within the cell.

In conclusion, we present a novel function for an antimicrobial peptide. PR-39, a peptide which is secreted into the extracellular environment, can directly interact with cell membranes, penetrate cells, and affect intracellular signal transduction factors. This adds to the growing list of functions already assigned to PR-39 alone. To elucidate a mechanism of antimicrobial action of PR-39 has not been explained by classical pore-forming models used to describe killing by most antimicrobial agents. PR-39 Binds and Affects p130 Cas

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