JNK1 and JNK2 Oppositely Regulate p53 in Signaling Linked to Apoptosis Triggered by an Altered Fibronectin Matrix

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The extracellular matrix regulates many cellular processes, including survival, and alterations in the matrix or in matrix survival signals can trigger apoptosis. Previously, we showed that an altered fibronectin matrix triggers apoptosis in primary cells via a novel pathway regulated by transcriptionally mediated decreases in p53 and c-Myc levels. Here we report that this apoptotic mechanism is propagated by decreased phosphorylation of focal adhesion kinase (FAK), which is linked to increased phosphorylation of c-Jun N-terminal kinase (JNK) and to decreased levels of p53. FAK is physically and spatially linked to JNK and p53, which relocalize from the nucleus to the cell membrane to mediate this interaction. Further, p53 participates in a feedback mechanism with JNK to regulate this apoptotic process and is oppositely regulated by JNK1 and JNK2.

Alterations in the extracellular matrix during inflammation, development, or metastatic processes can alter the cellular biology that governs these processes. Under these conditions, an altered matrix can result from proteolytic cleavage or alternative splicing of matrix molecules. In inflammation, proteolysis of extracellular matrix molecules leads to the formation of fragments, such as fibronectin (FN)1 fragments, that elicit cellular responses different from those elicited by the intact molecule (1–5). For example, disease-associated fragments of FN trigger p53-mediated apoptosis of primary cells, but the intact molecule does not (6–8). Similarly, alternatively spliced variants of FN elicit different cell responses, and specific domains of FN regulate cell survival (4, 5).

Previously we reported that an altered FN matrix triggers apoptosis in primary cells via a novel pathway that is regulated by transcriptionally mediated decreases in p53 and c-Myc levels in primary cells (5). To further decipher this apoptotic pathway, we explored the possible connections between integrin/FAK-mediated signals and the down-regulation of p53. We hypothesized that c-Jun N-terminal kinase (JNK) might be at the crossroads of this signaling pathway, since FAK and associated focal adhesion proteins (Racl/Fak1/MK4/JNK) have been linked to p53 status in the apoptosis of primary cells (9, 10). In addition, JNK can phosphorylate (11) and form a complex with p53 to stabilize it (12–16), thereby influencing its activity directly.

FAK is an integrin-associated protein tyrosine kinase that is important in integrin signaling. Its activation, triggered by increased phosphorylation of Tyr397 and other sites, has been implicated in many cellular processes, including cell survival. The N-terminal domain of FAK directs interactions with integrins and growth factor receptors. FAK also has a central catalytic domain, which contains Tyr397, a major autophosphorylation site and a site of interaction with the Src homology 2 domain. Its C-terminal noncatalytic domain, also known as FAK-related non-kinase (FRNK), contains sites for multiple protein-protein interactions and a focal adhesion targeting (FAT) region. Both FRNK and FAT act in a dominant-negative fashion to inhibit cell spreading and migration and growth factor-mediated signals to mitogen-activated protein kinase (17).

JNK, a group of mitogen-activated protein kinases that are activated by cytokines or environmental stress, participate in various signaling pathways, including apoptotic pathways. JNK proteins are encoded by three genes, JNK1 (46 kDa), JNK2 (54 kDa), and JNK3 (54 kDa), each of which undergoes alternative splicing to express various isoforms. JNK is activated by phosphorylation of specific threonine and tyrosine residues by mitogen-activated protein kinase kinases (18). Activated JNK1 can modify p53 posttranslationally by phosphorylation. JNK2 and JNK3 can also phosphorylate p53, and all three associate with p53 in vivo (19).

In this study, we sought to determine whether FAK signaling pathways communicate with p53 via JNK interactions in an altered matrix environment. Here we report that the apoptotic mechanism triggered by an altered FN matrix is propagated by decreases in FAK phosphorylation that are linked to increased phosphorylation of JNK and to decreased levels of p53, which is oppositely regulated by JNK1 and JNK2.

**EXPERIMENTAL PROCEDURES**

**Fibroblast Cell Culture**—Primary human fibroblasts were obtained, cultured, and tested for apoptosis as described (14). The use of human PDL (periodontal ligament) cells for these studies was approved by the University of California, San Francisco, Institutional Review Board, Committee on Human Research. p53-deficient and wild-type mouse

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fibroblasts were provided by Dr. Caroline Damsky (University of California, San Francisco) (10). jnk2-deficient mouse synoviocytes and wild-type controls were provided by Dr. Gary S. Firestein (University of California, San Diego School of Medicine). In addition, jnk2-deficient fibroblast cells and wild-type controls and jnk1/jnk2-deficient and wild-type control fibroblasts obtained from the respective knock-out mice and controls were provided by Dr. Roger Davis (Howard Hughes Medical Institute, University of Massachusetts Medical School, Worcester, MA).

Plasmids/DNA Constructs—The FAK, FAT, and FRNK constructs were provided by Dr. Caroline Damsky and Dr. Dusko Ilic (University of California, San Francisco). Constructs encoding JNK1, JNK2, and a dominant-negative, catalytically inactive JNK1 mutant (in which Thr183 and Tyr185 were replaced by Ala183 and Phe185) were provided by Dr. Roger Davis. Human wild-type p53 expression plasmid pC53-SN3 was provided by Bert Vogelstein (John Hopkins University School of Medicine, Baltimore, MD) (20).

Oligonucleotides—The oligonucleotides used for these experiments are shown in Table 1.

Recombinant FN Proteins—Two recombinant FN fragments were tested in these experiments. These fragments, described elsewhere (3), included the alternatively spliced V region (V/H11001) and contained either an unmutated (H/H11001) or a mutated, nonfunctional (H/H11002) high affinity heparin-binding domain.

Western Blot Analysis—For Western blot analysis, cells were incubated with the V/H11001-H/H11002 fragment, the V/H11001-H/H11001 fragment, or control serum-free medium and lysed with 100 µl/well TNE buffer (1% Nonidet P-40, 10% glycerol, 150 mM sodium chloride in Tris, pH 7.4, and 1 mM EDTA) containing protease inhibitors (1 mM sodium orthovanadate, 50 µM sodium molybdate, 25 µg/ml aprotinin, 25 µg/ml leupeptin, 1 mM sodium fluoride, and 1 mM phenylmethylsulfonyl fluoride) and phosphatase inhibitor mixtures 1 and 2 (Sigma). Lysates were adjusted for protein concentration with the BCA protein assay kit (Pierce), resolved

**TABLE I**

| Name | Sequence | Strand |
|------|----------|--------|
| FAK  | 5′-TTTCAACCAGATGCATTTC-3′ | Antisense |
| Scr FAK | 5′-TTTCAACCAGATGCATTTC-3′ | Antisense |
| JNK1 | 5′-CTCTCTGTGGGCCCTGATG-3′ | Antisense |
| JNK1a | 5′-CTCATGATGGCAAGCAATTA-3′ | Antisense |
| Scr JNK1 | 5′-CTTTCCTGTGGAGCCTGAGG-3′ | Antisense |
| JNK2 | 5′-GTCCGGCGCAGCCAAAGTGC-3′ | Antisense |
| JNK2a | 5′-GTCGAGTGGAGCATGGAAG-3′ | Antisense |
| Scr JNK2 | 5′-GTGGCCCGCGAGCGATATC-3′ | Antisense |

* Second additional antisense oligonucleotide.

**FIG. 1.** An altered FN matrix activates a signaling pathway that involves FAK, JNK, and p53. A, Western blots of cell lysates showing that the V′H″ FN fragment decreased FAK phosphorylation at Tyr397, increased JNK phosphorylation, and decreased total p53 levels in primary human fibroblasts compared with cells incubated in control medium (C) or with the control V′H″ FN fragment for 1, 3, and 7 h. Actin served as a loading control. B, after immunoprecipitation (IP) of the samples shown in A with an antibody against pJNK, Western blotting with primary antibodies against p53 and pFAK (Tyr397) pulled down p53 and pFAK, respectively, consistent with complex formation between pJNK and p53 and between pJNK and pFAK. C, fluorescence images showing the localization of FAK, pJNK, and p53 in primary human fibroblasts treated with the V′H″ protein or control medium (CTL) for 6 h. Cells in the top row were labeled first with primary antibodies against FAK, pJNK, and p53, then with secondary biotinylated antibodies, and finally with FITC-conjugated streptavidin. Cells in the bottom row were stained with DAPI nuclear stain. Original magnification, ×400.
FAK overexpression suppresses JNK phosphorylation and increases p53 levels, whereas dominant-negative constructs FRNK and FAT and FAK antisense treatment increase JNK phosphorylation and decrease p53 levels under altered matrix conditions. The diagram at the top depicts the three FAK domains, the N-terminal, catalytic, and C-terminal domains, and two of the major phosphorylation sites (Y397 and Y925). It also illustrates that the C-terminal domain is known as the FRNK domain, which contains the FAT region. A. Western blots illustrate the levels of FAK, pFAK (phosphorylated at Tyr397 or Tyr925), pJNK, and p53 in primary human fibroblasts transfected with FAK, FRNK, FAT or a vector control and treated with the V'-FN fragment for 1, 3, and 7 h. C, untreated control. Actin served as a loading control. B. Western blots of cells transfected with an antisense FAK oligonucleotide (AS FAK), a scrambled FAK oligonucleotide control (Scr FAK), or a vector control (CTL) and treated as described in A.

Immunofluorescence—Fluorescence staining was used to localize FAK, JNK, and p53 in cells incubated with the recombinant FN proteins or with control medium for 6 h. After incubation, cells were fixed with a 3% paraformaldehyde solution in phosphate-buffered saline (PBS), pH 7.2–7.6, and permeabilized with acetone at 20 °C. Nonspecific staining was blocked with a biotin-avidin blocking kit (Vector Laboratories). Cells were then incubated with a primary antibody against p53 (DO-1, anti-mouse antibody), pJNK (G-7 anti-mouse antibody), and FAT (G-2 anti-rabbit antibody) overnight, washed with PBS, incubated with secondary biotinylated antibodies, washed with PBS, and finally incubated with fluorescein isothiocyanate (FITC)-conjugated streptavidin. All antibodies were from Santa Cruz Biotechnology or Amersham Biosciences.

Nuclear staining of DNA was used to assess the quality of the nucleus in cells incubated with the recombinant FN proteins or with control medium in 16-well chamber slides for 6 or 14 h. After incubation, cells were fixed with ice-cold 100% methanol for 15 min, stained with a fluorescent groove-binding probe for DNA, 4',6-diamidino-2-phenylindole (DAPI, Sigma), for 10 min, rinsed three times with calcium- and magnesium-free PBS, dried, and sealed with a coverslip and mounting medium. Cells were photographed at ×400 magnification with a Nikon E400 photomicroscope equipped with a DAPI fluorescence filter, and imported into Photoshop 5.0.3.

Immunoprecipitation—For immunoprecipitation analysis, cells were incubated with the V'-FN fragment, the V''-FN fragment, or control serum-free medium and lysed with TNE buffer (100 μl/well) containing the protease inhibitors mentioned above. Lysates were adjusted for protein concentration with the BCA protein assay kit (Pierce), pre-cleared with protein G (Amersham Biosciences), incubated with pJNK (Santa Cruz Biotechnology), and protein G-9 overnight, pelleted by centrifugation, resuspended in lysis buffer, boiled for 5 min, and pelleted by centrifugation. The supernatants were resolved by SDS-PAGE, transferred to Immobilon-P membranes, probed with primary antibodies against p53 (Santa Cruz Biotechnology) and pFAK (BIO-SOURCE), and developed with ECL.

Antisense Experiments—To assess the effect of down-regulating the expression of FAK and JNK, we used the antisense oligonucleotides listed in Table I. The probes were synthesized by Oligos Etc. (Wilsonville, OR) as described (21, 22), except that the oligonucleotides were phosphorothioated at all positions to minimize intracellular cleavage by degradative enzymes and to enhance their stability (23) and were purified by high-performance liquid chromatography (level 1). For each antisense oligonucleotide, we also synthesized its sense oligonucleotide as a specific and relevant control. All oligonucleotides were dissolved in double distilled water and stored in aliquots at −20 °C.

Oligonucleotides (final concentration, 2 μM) were mixed with 8 μl of Oligofectamine (Invitrogen), incubated for 20 min, and transfected into cells at 60% confluence. Then cells were typically cultured in serum-free medium with the different oligonucleotides for 4 h at 37 °C; serum was added, and the cells were incubated for 48 h. After removal of the serum, the cells were washed with medium, pretreated with lactulamin hydrolysate (1 ml/well, Invitrogen), and incubated with FN proteins or with control medium in 16-well chamber slides for 6 or 14 h. After incubation, cells were fixed with a 3% paraformaldehyde solution in phosphate-buffered saline (PBS), pH 7.2–7.6, and permeabilized with acetone at −20 °C. Nonspecific staining was blocked with a biotin-avidin blocking kit (Vector Laboratories). Cells were then incubated with a primary antibody against p53 (DO-1, anti-mouse antibody), pJNK (G-7 anti-mouse antibody), and FAT (G-2 anti-rabbit antibody) overnight, washed with PBS, incubated with secondary biotinylated antibodies, washed with PBS, and finally incubated with fluorescein isothiocyanate (FITC)-conjugated streptavidin. All antibodies were from Santa Cruz Biotechnology. After washing, cells were dried, mounted on glass slides, and examined with a Nikon E400 photomicroscope equipped with a FITC fluorescence filter, and imported into Photoshop 5.0.3.

Nuclear Staining—Nuclear staining of DNA was used to assess the quality of the nucleus in cells incubated with the recombinant FN proteins or with control medium in 16-well chamber slides for 6 or 14 h. After incubation, cells were fixed with ice-cold 100% methanol for 15 min, stained with a fluorescent groove-binding probe for DNA, 4',6-diamidino-2-phenylindole (DAPI, Sigma), for 10 min, rinsed three times with calcium- and magnesium-free PBS, dried, and sealed with a coverslip and mounting medium. Cells were photographed at ×400 magnification with a Nikon E400 photomicroscope equipped with a DAPI filter.

RESULTS

An Altered Fibronectin Matrix Activates a Signaling Pathway That Involves FAK, JNK, and p53—To examine the possibility that JNK might be at the intersection of FAK- and p53-mediated signals in apoptosis triggered by an altered FN matrix, we assessed protein expression levels in primary hu-
man fibroblasts treated with FN fragments containing the alternatively spliced V region (V$^+$) and either a mutated (H$^-$) or a wild-type (H$^+$) heparin-binding domain (Fig. 1A). In cells treated with the V$^+$H$^-$ fragment, previously shown to induce apoptosis (18, 19), phosphorylation of JNK increased in tandem with decreases in p53 and FAK phosphorylation. FAK was phosphorylated at Tyr397 but not at Tyr925. Total FAK and JNK protein levels were not altered.

To determine whether JNK is physically linked to these signaling molecules, we performed immunoprecipitation and immunofluorescence localization experiments in primary human fibroblasts. An antibody against phosphorylated JNK (pJNK) immunoprecipitated both p53 and pFAK under control conditions. However, upon treatment with V$^+$H$^-$ FN fragment, these associations decreased with time (Fig. 1B); moreover, FAK localized to the cell membrane, and JNK and p53 relocalized from the nucleus to the cell membrane (Fig. 1C), indicating that FAK, JNK, and p53 are spatially coincident. The nuclei of cells treated with V$^+$H$^-$ appeared pyknotic and condensed, indicating apoptosis, whereas the nuclei of control cells were large and oval and not apoptotic (Fig. 1C). These findings suggest that in this apoptotic mechanism triggered by an altered matrix, JNK and p53 form a signaling complex that interacts with integrin/membrane/FAK signaling molecules.
FAK Is an Upstream Regulator of JNK Phosphorylation and p53 Protein Levels—To examine how changes in FAK expression might affect JNK and p53 signals, we transiently transfected cells with FAK cDNA, dominant-negative deletion mutants of FAK, FAT, and FRNK, or a FAK antisense oligonucleotide. Cells transfected with FAK and treated with the proapoptotic FN fragment expressed lower levels of pJNK and higher levels of p53 than controls, whereas cells transfected with the FAT, FRNK, or FAK antisense constructs expressed higher levels of pJNK and lower levels of p53 than controls (Fig. 2). As expected, the level of phosphorylation of FAK at Tyr^397 was sustained in FAK-transfected cells but not in cells transfected with FRNK or FAT. Studies in cells transfected with empty vector or scrambled oligonucleotides were performed to control for nonspecific alterations in protein or phosphorylation levels. These studies showed that JNK and p53 act downstream of FAK and that phosphorylation of Tyr^397 in the FAK catalytic domain is likely important in this pathway.

JNK1 and JNK2 Are Opposite Regulators of p53—To further examine the association of JNK with FAK and p53 signals in apoptosis triggered by an altered matrix, we performed a series of experiments in cells transfected with JNK1 or JNK2 cDNA, and p53 in primary human fibroblasts transfected with JNK2 or vector control or mock transfected and treated with the V^H^-FN fragment for 1, 3, and 7 h. C, untreated controls. Actin served as a loading control.

**FIG. 4.** Antisense JNK1 treatment increases p53 levels. A, Western blots showing the levels of JNK1, FAK, pFAK (phosphorylated at Tyr^397), pJNK, and p53 in primary human fibroblasts cells transfected with an antisense JNK1 (AS JNK1) oligonucleotide or a scrambled JNK1 oligonucleotide (Scr JNK1) or mock transfected and treated with the V^H^- fragment for 1, 3, and 7 h. C, untreated controls. Actin served as a loading control. B, Western blots showing JNK1, pJNK, and p53 levels in primary human fibroblasts transfected with a different antisense JNK1 oligonucleotide (AS JNK1*), a scrambled JNK1 oligonucleotide (Scr JNK1) or mock transfected and treated with the V^H^- fragment for 1, 3, and 7 h. Actin served as a loading control.

**FIG. 5.** Overexpression of JNK2 does not suppress p53 protein levels. Western blots showing the levels of JNK2, pJNK, JNK1, FAK, pFAK (phosphorylated at Tyr^397), and p53 in primary human fibroblasts transfected with JNK2 or vector control or mock transfected and treated with the V^H^-FN fragment for 1, 3, and 7 h. C, untreated controls. Actin served as a loading control.

**FIG. 6.** Antisense JNK2 treatment decreases p53 protein levels. A, Western blots showing the levels of JNK2, FAK, pFAK (phosphorylated at Tyr^397), pJNK, and p53 in primary human fibroblasts transfected with an antisense JNK2 oligonucleotide (AS JNK2) or a scrambled JNK2 oligonucleotide (Scr JNK2) or mock transfected and treated with the V^H^-FN fragment for 1, 3, and 7 h. C, untreated controls. Actin served as a loading control. B, Western blots showing JNK2, pJNK, and p53 levels in primary human fibroblasts transfected with a different antisense JNK2 oligonucleotide (AS JNK2*) or a scrambled JNK2 oligonucleotide (Scr JNK2) or mock transfected and treated with the V^H^-FN fragment for 1, 3, and 7 h. Actin served as a loading control.
a dominant-negative, catalytically inactive construct of JNK1, or antisense oligonucleotides for JNK1 or JNK2. In JNK1-overexpressing cells treated with the proapoptotic FN fragment, p53 decreased more than in control cells (Fig. 3A), and JNK phosphorylation increased. The levels of FAK, FAK phosphorylated at Tyr397, and actin were unchanged.

In cells transfected with a dominant-negative JNK1 construct, p53 levels increased, and JNK phosphorylation levels decreased (Fig. 7). Western blots showing the levels of FAK, pFAK (phosphorylated at Tyr397), pJNK, JNK2, and p53 in jnk2-deficient or control (CTL) cells treated with the V¹H–FN fragment for 1, 3, or 7 h. C, untreated controls. Actin served as a loading control.

FIG. 8. p53 can feed back on pJNK to decrease pJNK protein levels in this pathway. Overexpressing p53 leads to a greater decrease in pJNK levels, whereas knocking out the p53 gene leads to greater increases in pJNK protein levels in this pathway. A, Western blots showing the levels of p53, pJNK, JNK1, JNK2, FAK, and pFAK (phosphorylated at Tyr397) in primary human fibroblasts transfected with p53 or vector control or mock transfected and treated with the V¹H–FN fragment for 1, 3, and 7 h. C, untreated controls. Actin was used as a loading control. B, Western blots showing the levels of FAK, pFAK (phosphorylated at Tyr397), pJNK, JNK2, JNK1, and p53 in p53 knockout cells and wild-type controls treated with the V¹H–FN fragment for 1, 3, and 7 h. Actin served as a loading control.
FAK phosphorylation at Tyr397. Changes were noted in total FAK, JNK2, and actin levels or signals and blocking its ability to down-regulate p53. No JNK1 had been mutated, preventing its activation by upstream protein kinases. A double-ended arrow in Fig. 8 shows that FAK dephosphorylation leads to an increase in p53 protein levels (negative regulator), and p53 can feed back on JNK1 to negatively regulate JNK1 phosphorylation (double-ended arrow). JNK2 positively regulates p53. JNK and p53 relocalize (red arrows) from the nucleus to the cell membrane to interact with FAK to transmit the apoptotic signal.

DISCUSSION

Finally, we examined cells overexpressing or deficient in p53 and c-Myc levels in primary cells in response to an altered matrix. In this study, we demonstrate that this signaling mechanism is propagated by decreases in FAK phosphorylation, likely mediated by Tyr397, which are linked to increases in JNK phosphorylation and decreases in p53 protein levels. Furthermore, JNK1 and JNK2 had opposite effects in regulating p53, which appeared to participate in a feedback mechanism with JNK to facilitate this apoptotic process (Fig. 9).

Several studies have shown that JNK interacts with (13, 14) and can phosphorylate p53 (11). In fact, p53 and JNK can form a complex, and JNK is a regulator of p53 stability (13–16). In one study, sustained activation of JNK1 down-regulated p53 expression during apoptosis (16), similar to our findings. However, we found that JNK1 and JNK2 are opposite regulators of p53. Surprisingly, treatment with antisense JNK2 suppressed p53 levels in controls and under altered FN matrix conditions. JNK1 antisense treatment, however, increased p53 levels. P53 expression was also suppressed in jnk2-deficient cells. These data indicate that JNK2 expression is tightly linked to that of p53 and that JNK1 and JNK2 regulate p53 by different mechanisms. Furthermore, increases in JNK1 expression (or pJNK1) decreased p53 protein levels, and decreases in JNK1 expression (or pJNK1) increased p53 levels. Conversely, increases in JNK2 expression (or pJNK2) may increase p53 levels, and decreases in JNK2 expression (or pJNK2) profoundly decreased p53 levels. Thus JNK1 is a negative regulator and JNK2 is a positive regulator of p53 expression.

The antibody we used to detect pJNK also detects JNK1, -2, and -3, and Western blots for pJNK showed two bands at ~54 and ~46 kDa. For several reasons, however, we are confident that treatment with an altered FN matrix increased JNK1 phosphorylation only and not that of JNK2 or JNK3. First, transfection with a JNK1 construct (Fig. 3A) and a dominant-negative JNK1 construct (Fig. 3B) both showed an increase in the two bands visible in the pJNK Western blots under control conditions, indicating that both bands are JNK1 isoforms. Second, treatment with a JNK1 antisense oligonucleotide decreased both bands, indicating that they are both JNK1 isoforms (Fig. 4A). Third, transfection with a JNK2 construct induced a new higher molecular mass band at ~54 kDa for pJNK that was not seen before with any other treatments and likely represents the phosphorylated form of JNK2 (Fig. 5). Fourth, Western analysis of jnk2-deficient cells showed two bands that likely represent two isoforms of JNK1. These bands were not seen in Western blots of cells deficient in JNK1 and JNK2.

Interestingly, overexpression of p53 limited the phosphorylation of JNK normally induced by an altered FN matrix, suggesting that p53 and pJNK participate in a negative feedback mechanism. Similarly, in previous studies, introduction of p53 into p53-null cells reduced JNK activity to a low basal level (24), possibly indicating feedback between p53 and JNK.

JNKs are a family of stress-related kinases, and an altered FN matrix represents a stressful condition in which JNKs seem to play a role. At sites of inflammation, the extracellular matrix is degraded by proteolytic enzymes in the inflammatory milieu. Degraded matrix conveys messages that are different from those normally conveyed by an intact or stable matrix, and the degradation increases with the severity of the disease. In periodontal disease and arthritis, FN fragments are most abundant in the most diseased sites (6, 25–30). Previously, we found that a proteolytic 40-kDa fragment of FN associated with the most advanced disease also induced apoptosis of periodontal ligament fibroblasts in vitro by down-regulating p53 and c-Myc (6, 7). This 40-kDa fragment, which is comparable with the recombinant FN fragment we tested here, induced apo-
ptosis of fibroblasts via a novel alternative pathway that involves decreases in FAK phosphorylation and down-regulation of p53 and c-Myc. This pathway appears to be triggered by cooperative interactions between integrins and a chondroitin sulfate proteoglycan (4, 5). From the present findings we can further add that this inflammation-associated apoptotic pathway also involves interactions between pFAK and JNK1 and -2 and their phosphorylated isoforms, which oppositely regulate p53 expression levels.

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