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SPARC Mediates Focal Adhesion Disassembly in Endothelial Cells Through a Follistatin-Like Region and the Ca^{2+}-Binding EF-Hand

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Abstract SPARC is a one of a group of extracellular matrix proteins that regulate cell adhesion through a loss of focal adhesion plaques from spread cells. We previously reported that SPARC reduced the number of bovine aortic endothelial (BAE) cells positive for focal adhesions [Murphy-Ullrich et al. (1991): J Cell Biol 115:1127–1136]. We have now characterized the effect of SPARC on the cytoskeleton of BAE cells. Addition of SPARC to spread BAE cells caused a dose-dependent loss of focal adhesion-positive cells, that was maximal at ~1 μg/ml (0.03 μM). Consistent with the loss of adhesion plaques as detected by interference reflection microscopy, vinculin appeared diffuse and F-actin was redistributed to the periphery of cells incubated with SPARC. However, the distribution of the integrin $\alpha_v\beta_3$ remained clustered in a plaque-like distribution. These data, and the observation that SPARC binds to BAE cells but not to the extracellular matrix, indicate that SPARC acts via interactions with cell surface molecules and not by steric/physical disruption of integrin-extracellular matrix ligands. To determine the region(s) of SPARC that mediate a loss of focal adhesions, we tested peptides from the four distinct regions of SPARC. The cationic, cysteine-rich peptide 2.1 (amino acids 54–73) and the Ca^{2+}-binding EF-hand-containing peptide 4.2 (amino acids 254–273) were active in focal adhesion disassembly. Furthermore, antibodies specific for these regions neutralized the focal adhesion-labilizing activity of SPARC. These results are consistent with previous data showing that peptide 2.1 and 4.2 interact with BAE cell surface proteins and indicate that the loss of focal adhesions from endothelial cells exposed to SPARC is a receptor-mediated event. © 1995 Wiley-Liss, Inc.

Key words: SPARC, endothelial cell, focal adhesions, vinculin, integrin, cell adhesion

The regulation of cell-matrix interactions and cell shape is essential for control of a variety of cellular processes such as mitosis, migration, and tissue morphogenesis. It is now recognized that cell shape, as influenced by the cytoskeleton, and cell-matrix interactions, as mediated by integrin and proteoglycan interactions with extracellular matrix molecules, affect nuclear events such as cell cycle progression and gene expression [Ingber, 1993; Juliano and Haskill, 1993; Sims et al., 1992]. Specialized submembranous assemblies of cytoplasmic components called focal adhesion plaques link the cytoskeleton to the extracellular matrix through integrin/proteoglycan receptor systems. Focal adhesions are potential foci for the transmission of signals from the outside to the inside of the cell [Burridge et al., 1988; Woods and Couchman, 1988; Lo and Chen, 1994]. Phosphorylation/dephosphorylation of regulatory components in focal adhesion structures, such as talin, integrins, paxillin, and $pp125^{FAK}$, is thought to function in the control of cytoskeletal structure [Turner et al., 1989; Turner, 1991; Schaller et al., 1992; reviewed in Hynes, 1992; Juliano and Haskill, 1993].

A group of antiadhesive extracellular matrix proteins that control cytoskeletal organization and the integrity of focal adhesions has recently been identified [reviewed in Sage and Bornstein, 1991]. These proteins, e.g., thrombospondin

Abbreviations used: BAE, bovine aortic endothelial cells; DMEM, Dulbecco's modified Eagle's medium; IR, interference reflection; rSPARC, recombinant SPARC.

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[Murphy-Ullrich and Höök, 1989; Murphy-Ullrich et al., 1993], tenascin [Murphy-Ullrich et al., 1991; Spring et al., 1989], and SPARC [Sage et al., 1989a; Murphy-Ullrich et al., 1991], induce disassembly of focal adhesion structures from a subpopulation of spread endothelial cells and fibroblasts. The changes are readily characterized by a loss of vinculin from plaques and a cortical redistribution of actin-containing stress fibers. Although thrombospondin, tenascin, and SPARC are structurally distinct, a commonality is their enhanced expression by migrating, dividing cells (e.g., in wounds, during embryogenesis, and in subconfluent cultures) relative to stationary, quiescent cells [reviewed in Bornstein, 1992; Lahav, 1993; Erickson and Bourdon, 1989; Sage et al., 1989c]. In addition, each of these three proteins has been shown to inhibit cell attachment under certain conditions [Lahav, 1988; Murphy-Ullrich and Höök, 1989; Chiquet-Ehrismann et al., 1988; Spring et al., 1989; Sage et al., 1992]. The temporal expression of these proteins is consistent with the fact that focal adhesions are disassembled in migrating cells and reform when cells become stationary [Couchman and Rees, 1979]. Thus, this group of antiadhesive matrix glycoproteins has been implicated in control of cytoskeletal organization and the corresponding fidelity of cell-matrix interactions. Furthermore, these proteins can potentially function in the regulation of angiogenesis, cell proliferation, and tumor cell metastasis [Sage and Bornstein, 1991; Lahav, 1993; Bornstein, 1992; Frazier, 1991; Erickson and Bourdon, 1989].

SPARC (also known as osteonectin and BM-40) [see review by Lane and Sage, 1994] is an acidic, calcium-binding glycoprotein secreted by endothelial cells in response to culture shock [Sage et al., 1986; Mason et al., 1986]. SPARC induces endothelial cell rounding with reorganization of actin stress fibers and also prevents cell spreading [Sage et al., 1989a]. SPARC is widely expressed in embryonic tissues and may play a role in pattern formation [Holland et al., 1987; Sage et al., 1989b]. In the adult, expression of SPARC is high in remodeling and renewing tissues that are characterized by cells undergoing changes in shape (e.g., proliferation, migration, and wound repair) [Reed et al., 1993; Sage et al., 1989c]. SPARC also inhibits endothelial cell proliferation through a delay in cell-cycle progression from G1 to S phase [Funk and Sage, 1993], alters the expression of matrix and protease inhibitor genes [Lane et al., 1992; Tremble et al., 1993], increases the permeability of endothelial cell monolayers [Goldblum et al., 1994], and modulates growth factor activity, such as bFGF-induced chemotaxis [Hasselaar and Sage, 1992]. SPARC also interacts with interstitial collagens [Sage et al., 1989a; Lane and Sage, 1990] and binds the B-chain of platelet-derived growth factor [Raines et al., 1992]. In an effort to integrate these apparently diverse functions in the context of angiogenesis, we have proposed that the enhanced expression of SPARC during the formation of endothelial cords in vitro facilitates a decrease in cell adhesion through at least four mechanisms: (1) stimulation of cell rounding, (2) interaction with growth factors, (3) modulation of the cell cycle, and (4) diminution of the expression of adhesive extracellular matrix proteins [Iruela-Arispe et al., 1991; Lane et al., 1992; Lane and Sage, 1994].

In previous work, we reported that SPARC was associated with a loss of focal adhesions from spread endothelial cells [Murphy-Ullrich et al., 1991]. We now report that SPARC induces a dose-dependent loss of focal adhesions, with a corresponding redistribution of vinculin and actin stress fibers, but not of the integrin $\alpha_v\beta_3$. Furthermore, through the use of synthetic peptides, we have identified two regions within SPARC that mediate focal adhesion disassembly. These regions have recently been identified by the use of synthetic peptides as sequences mediating binding of SPARC to endothelial cell membrane proteins [Yost and Sage, 1993]. One of the functions of these putative receptors might therefore be to signal changes in the integrity of adhesion structures.

MATERIALS AND METHODS

Materials

The following materials were purchased: Dulbecco's modified Eagle's medium (DMEM) (Cell-Gro, Mediatech, Herndon, VA); fetal bovine serum (Hyclone Laboratories, Logan, UT); 500 μg/ml trypsin, 2.2.mM EDTA (Life Technologies, Inc., Grand Island, NY); and bovine serum albumin, glutaraldehyde (Sigma Chemical, St. Louis, MO). Rabbit anti-human tenascin was purchased from Telios Pharmaceuticals (La Jolla, CA) and the IgG fraction was purified with Protein A as described [Murphy-Ullrich et al., 1991].

Cells

Bovine aortic endothelial (BAE) cells were isolated and cultured in DMEM containing 4.5 g/liter glucose, 2 mM glutamine, and 20% fetal bovine serum as previously described [Murphy-Ullrich et al., 1993].

SPARC Purification

Mouse SPARC was purified from the conditioned medium of mouse parietal yolk sac (PYS-2) cells as described [Sage et al., 1989a]. Recombinant murine SPARC (rSPARC) was expressed in *Saccharomyces cerevisiae* (Yost et al., manuscript submitted). This rSPARC contained no N-linked carbohydrate, since Asn_{198} was mutated to Asp_{198}. An ammonium sulfate precipitate of proteins synthesized by the same strain of *S. cerevisiae,* transfected with expression vector but without the SPARC sequence, was used as a control.

Focal Adhesion Assay

Focal adhesion assays were performed as described [Murphy-Ullrich and Höök, 1989; Murphy-Ullrich et al., 1991] with the following modifications. BAE cells were grown on glass coverslips for ~24 h in the presence of either 20% or 5% fetal bovine serum until just confluent. Cell were pretreated for 1 h with 10 μg/ml cycloheximide, rinsed once with warm DMEM, and incubated with SPARC, SPARC peptides, or BSA for 1 h at 37°C in the continued presence of cycloheximide. Cells were subsequently fixed with 3% warmed glutaraldehyde for 30 min, washed, mounted on glass slides, and examined by interference reflection microscopy (IRM) with either a Nikon Optiphot microscope or a Zeiss Axiovert 10 microscope. A minimum of 200 cells/condition was counted. Cells having at least 3 adhesion plaques were designated as positive.

Immunofluorescence

Immunolocalization of vinculin, $\alpha_v\beta_3$ integrin, and SPARC was performed on formaldehyde-fixed and Triton-X-100 permeabilized cells exactly as described previously [Murphy-Ullrich and Höök, 1989]. Monoclonal anti-vinculin ascites fluid (clone VIN-11-5) was purchased from Sigma. Monoclonal anti-alpha$_v$ integrin chain (clone VNR147) was purchased from Life Technologies, Inc. Rabbit polyclonal anti-SPARC antiserum was produced as described [Sage et al., 1989]: to eliminate potential interference from serum factors, the IgG fraction was isolated with a Protein A-Sepharose column. Bodipy-phallicidin was purchased from Molecular Probes, Inc. (Eugene, OR). Rhodamine-conjugated goat anti-mouse or anti-rabbit IgG was purchased from Jackson Laboratories (West Grove, PA).

Peptides and Antipeptide Antibodies

Peptides of 20 amino acids corresponding to different regions of mouse SPARC were synthesized by Dr. Patrick Chou (Howard Hughes Medical Institute, University of Washington, Seattle, WA) and Kathy Walker (ZymoGenetics Corp., Seattle, WA). Peptides were purified and amino acid sequences were confirmed as described [Lane and Sage, 1990; Lane et al., 1992].

Rabbit polyclonal antibodies were produced against keyhole limpet hemocyanin-conjugated peptides as described by Lane and Sage [1990]. The IgG fraction was prepared by precipitation of the antiserum in 20% ammonium sulfate.

RESULTS

We had previously shown that thrombospondin and tenascin cause a rearrangement of the actin cytoskeleton and a loss of vinculin-containing focal adhesion plaques. Since incubation of enothelial cells with SPARC results in cell rounding or an inhibition of cell spreading after 4 h [Sage et al., 1989a], we examined whether SPARC might also destabilize cell adhesion through disassembly of focal adhesion plaques.

SPARC purified from murine cells was added to spread endothelial cells. After 1 h at 37°C, a loss of adhesion plaques from a subpopulation of these cells was apparent (Fig. 1). The extent of focal adhesion loss in the BAE cell population was simular to what was observed in cells treated with either thrombospondin or tenascin [Murphy-Ullrich et al., 1989, 1991]. A polyclonal rabbit IgG against SPARC inhibited the disassembly of focal adhesions by SPARC, whereas an antibody against tenascin had no effect (Fig. 1).

To exclude the possibility that the focal adhesion-disrupting activity of SPARC was due to trace impurities derived from the PYS cell conditioned medium, we tested rSPARC expressed in *S. cerevisiae* for antiadhesive activity. BAE cells treated with rSPARC showed a dose-dependent loss of focal adhesions with a maximal effect at ~1 μg/ml (0.03 μM) (Fig. 2). Endogenous proteins from *S. cerevisiae* had no effect on focal adhesion disassembly.

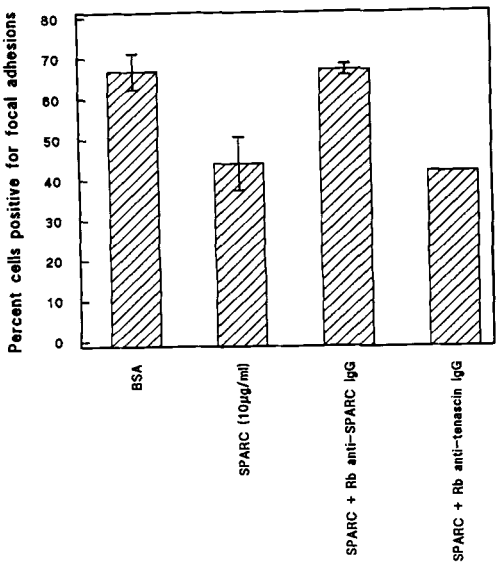

Fig. 1. Murine SPARC causes loss of focal adhesions from spread BAE cells. BAE cells were grown on coverslips in the presence of 20% fetal bovine serum in DMEM for 24 h, pretreated for 1 h with 10 μg/ml cycloheximide, washed in serum-free DMEM, and then incubated in the continued presence of cycloheximide for 1 h at 37°C with 10 μg/ml (0.3 μM) SPARC, SPARC + 50 μg/ml rabbit anti-SPARC IgG, SPARC + 50 μg/ml rabbit anti-tenascin IgG, or 7 μg/ml BSA. Cells were subsequently fixed and examined by IRM for the presence of positive cells (cells with greater than 3–5 adhesion plaques/cell). Results are expressed as the percent of cells positive for focal adhesions, with 200–300 cells examined/condition.

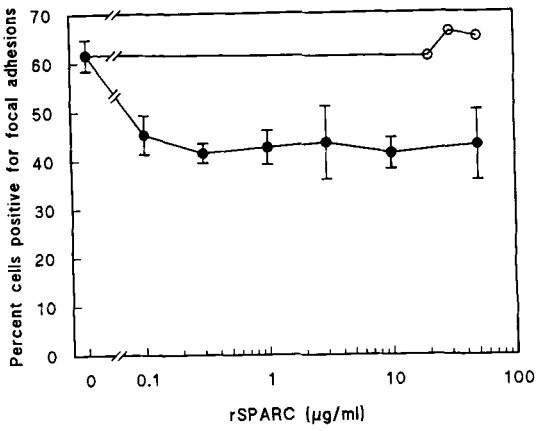

Fig. 2. Loss of focal adhesions is dependent on rSPARC concentration. BAE cells grown overnight on coverslips were incubated with increasing concentrations of rSPARC (closed circles) or with yeast proteins from yeast plasmid controls (open circles) for 1 h at 37°C as described in Materials and Methods. Cells with no added rSPARC were incubated with 7 μg/ml BSA. Cells were then examined for the presence of focal adhesions. Results are expressed as the percentage of cells positive for focal adhesions ± S.D.

Interference reflection (IR) images of SPARC-treated cells showed a loss of adhesion plaques and a general homogeneous gray appearance that was indicative of loss of tension on the basal membrane (Fig. 3). The distribution of vinculin in these cells corresponded to the IR images: specifically, the staining was diffuse throughout the cytoplasm of SPARC-treated cells in comparison to control cells, which displayed numerous adhesion plaques by IR and a plaque-like distribution of vinculin (Figs. 3, 4). The actin microfilaments were distributed in a peripheral web in SPARC-treated cells. The interaction of the integrin receptor ($\alpha_v\beta_3$) with its extracellular matrix ligand (presumably vitronectin) does not appear to be disturbed in cells treated with SPARC, since staining for the $\alpha_v\beta_3$ receptor retained its clustered distribution (Fig. 4). Similar results with respect to these proteins have been observed in cells treated with thrombospondin (data not shown).

Since SPARC did not appear to induce cytoskeletal reorganization by disruption of $\alpha_v\beta_3$ matrix interactions, SPARC might act through intracellular pathways resulting from its binding to the cell surface. Indeed, SPARC added to spread BAE cells appeared to be distributed over the cell surface, with some larger clusters of SPARC present at the cell edges and just proximal to the leading edge (Fig. 5). There was no detectable binding of SPARC to the endothelial cell matrix, a result consistent with previous reports (Fig. 5) [Sage et al., 1986, 1989a]. These data indicate that SPARC induces its effects on the cytoskeleton via interactions with endothelial cell receptors [Yost and Sage, 1993].

To determine which region of SPARC mediated the disassembly of focal adhesions, we tested peptides from the four distinct regions of SPARC in the focal adhesion assay (Table I). These studies showed that peptides 2.1 (amino acids 54–73) and 4.2 (amino acids 254–273) were associated with a loss of focal adhesion plaques, whereas peptide 3.2 (amino acids 154–173) had no effect in this assay (Fig. 6). Peptide 1.1 (amino acids 4–23) was associated with a partial decrease in focal adhesion-positive cells. Peptides 2.1 + 4.2, or 2.1 + 1.1 did not act additively at the concentrations tested, since there was no further loss of focal adhesions when mixtures of both peptides were incubated together with the cells. Moreover, peptide 1.1 did not block or enhance the effect of peptide 2.1 (data not shown). Antibodies against peptides 4.2 and 2.1 blocked the loss of focal adhesions that was

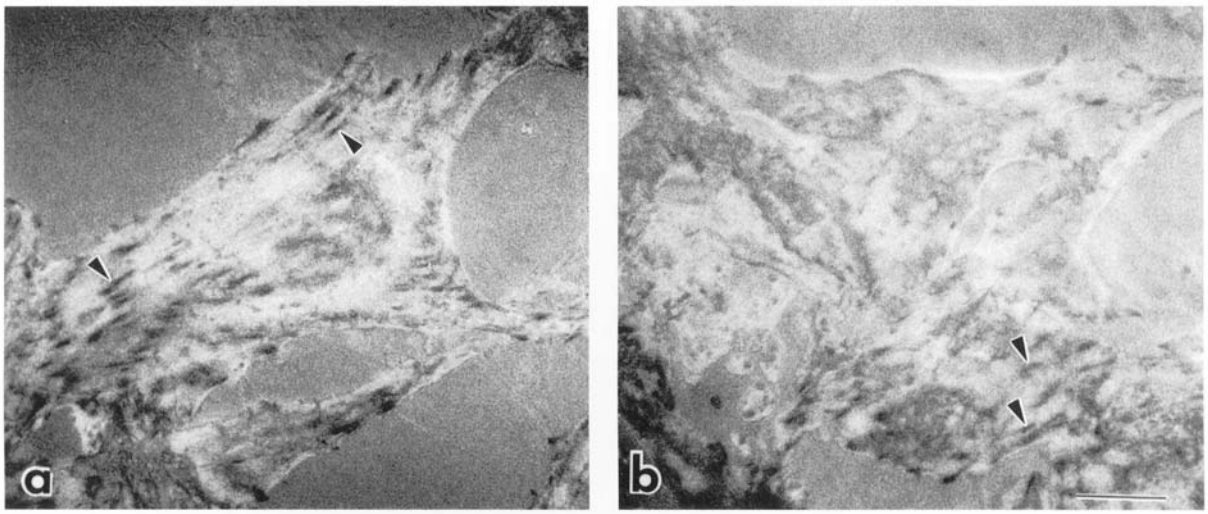

Fig. 3. Interference reflection images of cells treated with SPARC. BAE cells grown in the presence of serum were treated with cycloheximide, washed, and incubated with 10 μg/ml mouse SPARC or 6.7 μg/ml BSA for 1 h at 37°C. Subsequently, cells were fixed and examined for focal adhesions. BSA-treated control cells (**a**) had numerous adhesion plaques (arrowheads) distributed throughout the cytoplasm, whereas cells treated with SPARC (**b**) lacked adhesion plaques and exhibited a gray appearance, that is indicative of loss of tension at the cell basal membrane. Small plaques (arrowheads) are present in one of the cells in this field and there are at least three cells in this field that lack focal adhesions. Bar = 10 μm.

mediated by rSPARC (Fig. 7): samples treated with rSPARC in the presence of these anti-peptide antibodies had 90% of the number of cells positive for focal adhesions as compared to DMEM-treated control cells. Peptides from both of these domains competed for the binding of SPARC to endothelial cells [Yost and Sage, 1993].

DISCUSSION

Control of cell adhesion is essential for many processes during the life of a cell or tissue. Cell-matrix contacts are dynamic: forming, disassembling, and reforming in response to conditions which regulate mitosis, migration, and tissue morphogenesis. In particular, cells reorganize their cytoskeleton with a loss of focal adhesions and subsequent shape changes during wound healing, embryogenesis, angiogenesis, and metastasis. Tumor-promoting phorbol esters [Hedberg et al., 1990; Schliwa et al., 1984], certain growth factors [Herman and Pledger, 1985; Herman et al., 1986], and heart-cell conditioned medium [Dunlevy and Couchman, 1993] cause a loss of focal adhesions and reorganization of actin and vinculin from these structures. Components of the extracellular matrix also regulate cell adhesion. Although it has long been appreciated that certain matrix glycoproteins, such as fibronectin, vitronectin, and laminin, trigger cytoskeletal organization and promote cell-matrix interactions, only in recent years has it been recognized that matrix proteins also have destabilizing effects on cell adhesion [Murphy-Ullrich and Höök, 1989; Murphy-Ullrich et al., 1991, 1993; Spring et al., 1989; Chiquet-Ehrisman 1991; Sage et al., 1989a; Sage and Bornstein, 1991]. The anti-adhesive matrix proteins thrombospondin, tenascin, and SPARC, although structurally dissimilar, exhibit highly regulated expression and are present in elevated amounts in tissues with a high proportion of dividing and migrating cells.

Previous work showed that SPARC caused endothelial cell rounding after several hours. In the studies reported here, we show that SPARC also affects the earlier stages of the anti-adhesive process. Treatment of spread BAE cells with SPARC or SPARC peptides for 1 h resulted in a loss of vinculin-containing focal adhesion plaques, as observed by immunostaining and interference reflection microscopy, with concomitant reorganization of the actin-containing stress fibers. By IR, the morphology of the SPARC-treated cells was indistinguishable from that of cells exposed to thrombospondin or tenascin. Similarly, there was a subpopulation of endothelial cells refractory to the effects of SPARC, an observation previously reported with respect to the spreading of BAE cells [Sage et al., 1989a]. Cultures of both endothelial cells and fibroblasts also contain cells with adhesion plaques that are resistant to the effects of tenascin or

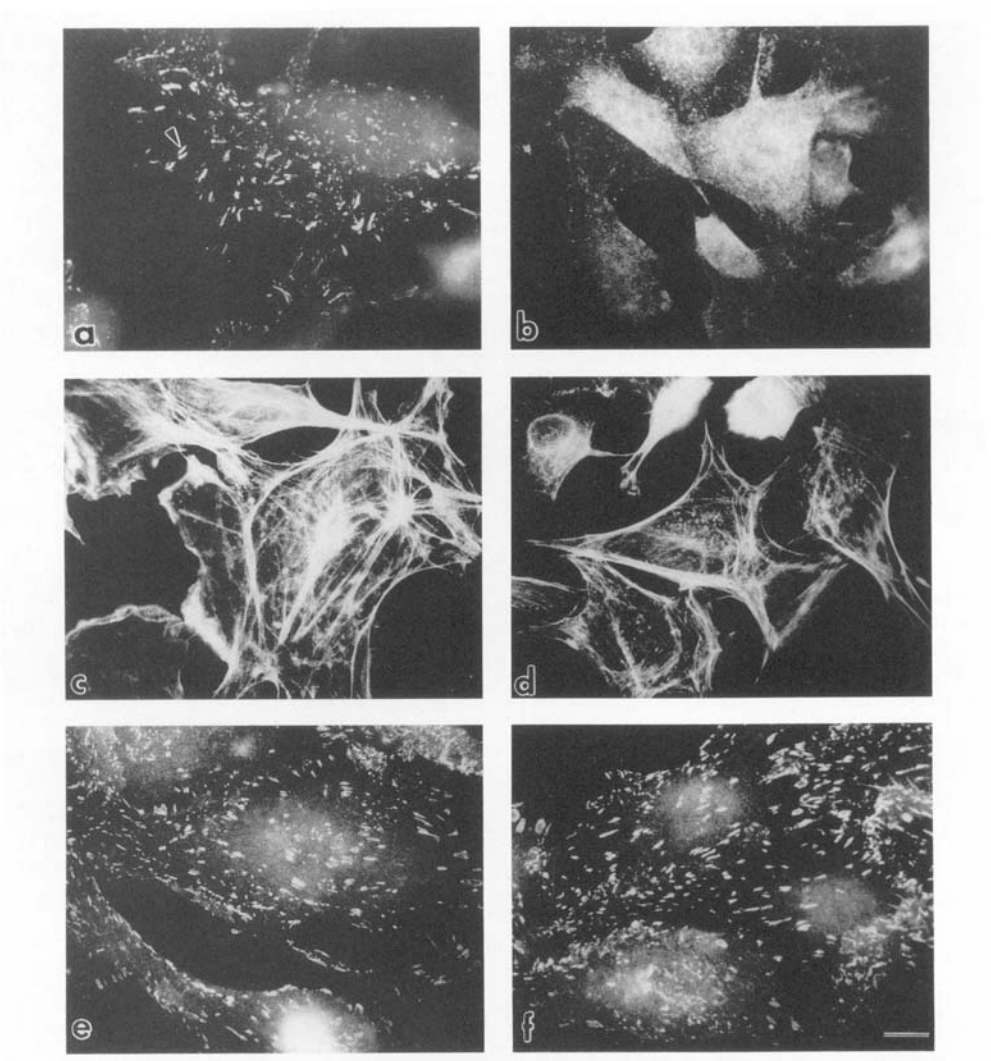

Fig. 4. The distribution of vinculin and F-actin, but not integrin, is altered in SPARC-treated cells. BAE cells were treated with murine SPARC (**b,d,f**) or BSA (**a,c,e**) as in Figure 3. Cells were then fixed and were stained with monoclonal antibody to vinculin (a,b), Bodipy-phallicidin to detect F-actin (c,d), or a monoclonal antibody to the α_v integrin subunit (e,f). BSA-treated control cells had numerous vinculin-containing plaques (a), abundant stress-fibers (c), and clusters of the $\alpha_v\beta_3$ integrin in plaques (e). SPARC-treated cells exhibited a primarily diffuse distribution of vinculin (b), and a peripheral rearrangement of F-actin (d). In contrast, the integrins containing the α_v chain remained clustered in a plaque-like pattern (f). Bar = 10 μm.

thrombospondin [Murphy-Ullrich and Höök 1989; Murphy-Ullrich et al., 1991] (and unpublished data). This phenomenon does not appear to be related to cell cycle, since synchronized cultures exhibit a similar number of refractory cells in the presence of these anti-adhesive proteins (unpublished data). We suspect that the structure of the adhesion plaques in the refractory population rather than the properties of the anti-adhesive proteins is a determining factor, since fibroblasts treated with heart-conditioned medium also exhibit a resistant subpopulation [Dunlevy and Couchman, 1993].

We found that cells treated with SPARC retained a plaque-like distribution of their integrin receptors despite the redistribution of actin and vinculin. Similar results were obtained with thrombospondin (data not shown). Several observations appear to be consistent with our data: (1) clustering of an integrin can precede that of cytoskeletal components during cell spreading [Dejana et al., 1988]; (2) clustering of β_1 integrin occurs in the presence of protein kinase C inhibitors that prevent the clustering of vinculin and organization of F-actin [Woods and Couchman, 1992]; and (3) PDGF-stimulated phosphorylation of talin in skeletal muscle cells results in a disassembly of F-actin stress fibers and a diffuse distribution of vinculin, although the β_1 integrin remains associated with focal adhesions

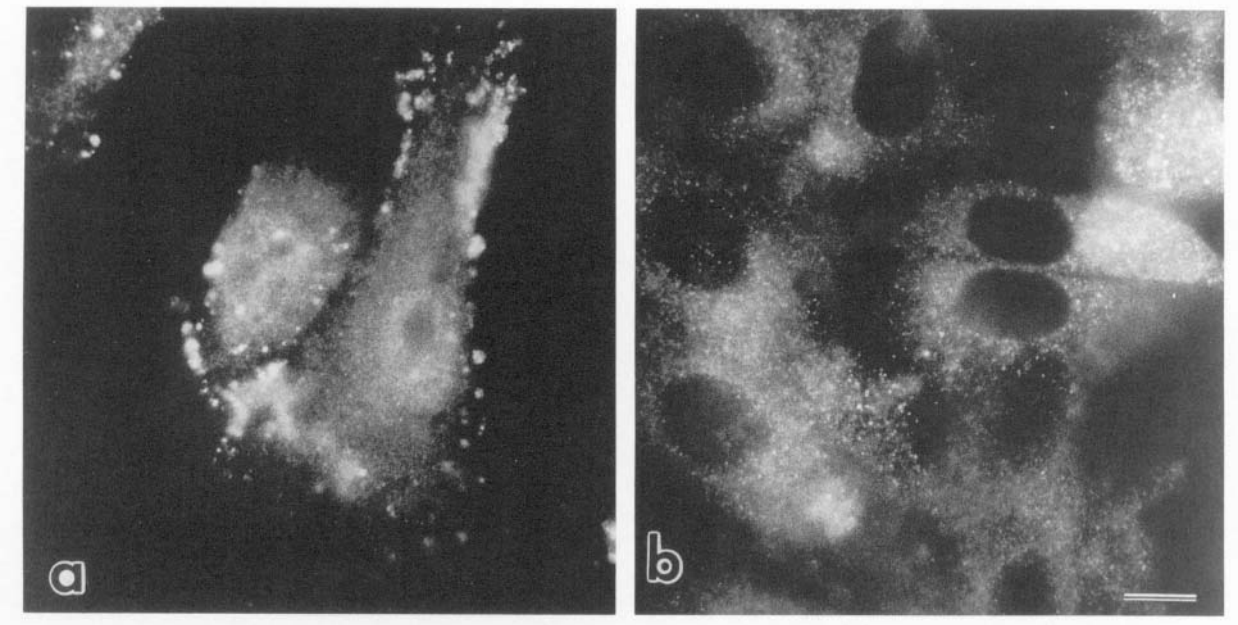

Fig. 5. Exogenous SPARC binds to BAE cells and not to the extracellular matrix. BAE cells grown overnight on coverslips were treated for 1 h at 37°C with either (**a**) 10 μg/ml rSPARC or (**b**) 7 μg/ml BSA in the presence of cycloheximide, fixed with paraformaldehyde, treated with 0.1% Triton-X 100, and immunostained with 10 μg/ml rabbit anti-SPARC antibody. rSPARC bound to the cells and was not detected in the extracellular matrix. Bar = 10 μm.

TABLE I. SPARC Peptides*

Peptide	Sequence	Sequence location
1.1	QTEVAEEIVE EETVVEETGV	5–23
2.1	CQNHHCKHG KVCELDESNTP	54–73
3.2	KNVLVTLYER DEGNNLLTEK	154–173
4.2	TCDLDNDKYI ALEEWAGCFG	254–273

*Sequences represent those of murine SPARC [Mason et al., 1986]. Numbers refer to position of amino acids in SPARC after removal of the signal sequence.

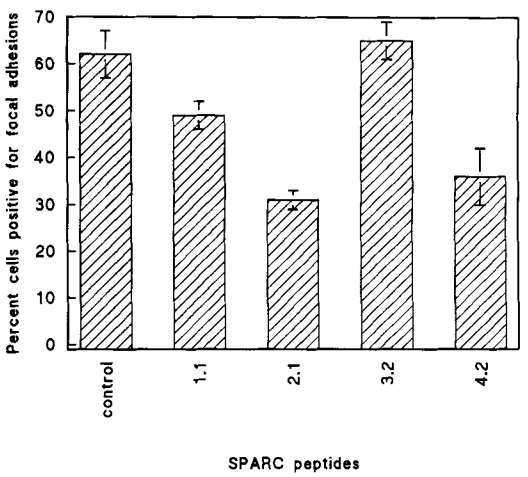

Fig. 6. SPARC peptides cause focal adhesion disassembly. BAE cells were grown on coverslips overnight, treated with cycloheximide, washed with serum-free DMEM, and incubated for 1 h at 37°C with either BSA or 0.2 mM SPARC peptides 1.1, 2.1, 3.2, or 4.2. Peptides 2.1 and 4.2 were associated with a loss of adhesion plaques, whereas peptide 3.2 had no effect, even at concentrations as high as 0.7 mM. Peptide 1.1 was associated with a partial loss of focal adhesions. Results are expressed as the mean percentage of cells positive for focal adhesions ± S.D. (n = 3–8).

[Tidball and Spencer, 1993]. In the context of these findings, we suspect that the primary effect of SPARC on adhesion, specifically in the initial stages of de-adhesion, is through the modulation of cytoskeletal components and not through a disruption of integrin-matrix ligand interactions. Therefore, it is reasonable to suggest that SPARC is acting through intracellular signals triggered by the binding of SPARC to its receptor(s). This would be consistent with evidence from several labs that protein kinase activity modulates cytoskeletal organization [Woods and Couchman, 1992; Hedberg et al., 1990; Lamb et al., 1988].

We showed that two distinct sequences of SPARC from the cysteine-rich, follistatin-like region (peptide 2.1) and the C-terminal, Ca^{2+}-binding EF hand (peptide 4.2) independently induced the disassembly of focal adhesions. The former, a cationic peptide from a non-Ca^{2+}-binding EF hand (peptide 4.2) independently induced the disassembly of focal adhesions. The

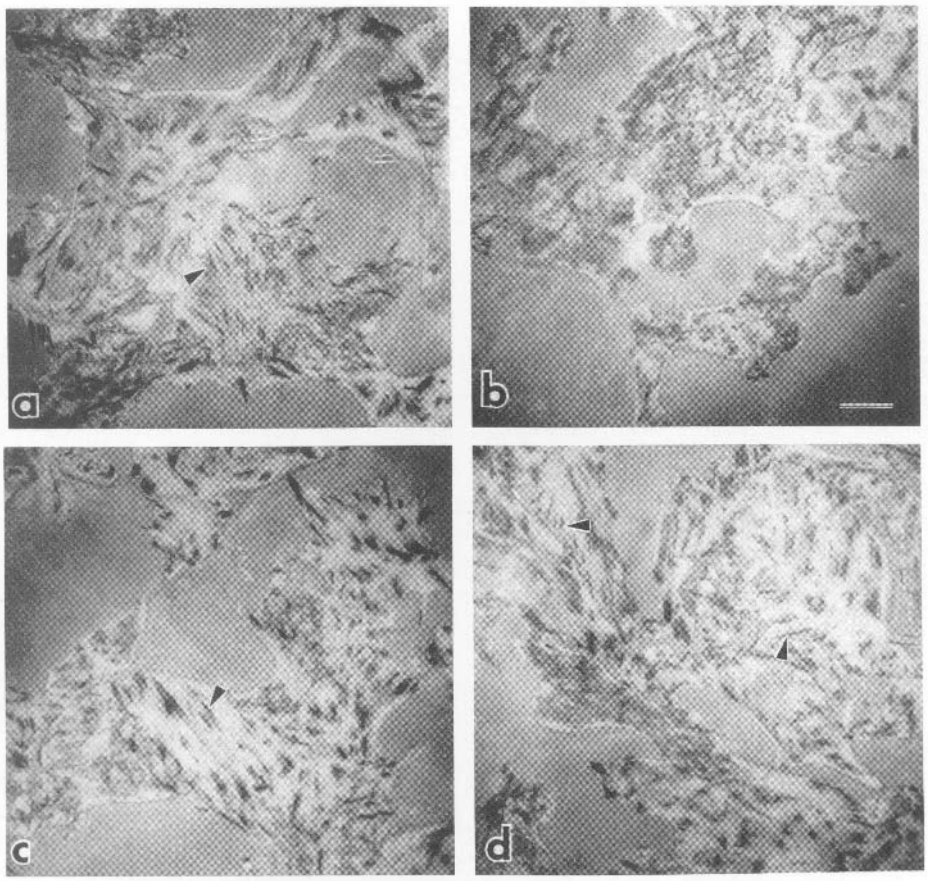

Fig. 7. SPARC anti-peptide antibodies prevent the loss of focal adhesions mediated by rSPARC. BAE cells were grown on coverslips overnight, washed, and treated with serum-free medium (control) **(a)**, 1 μg/ml rSPARC **(b)**, or rSPARC + 8 μg/ml rabbit antipeptide 2.1 **(c)**, or rSPARC + 250 μg/ml rabbit antipeptide 4.2 antibodies **(d)** for 1 h at 37°C as described in the text. Cells were subsequently fixed and examined for the presence of focal adhesions. Fifty-three percent of control cells incubated in serum-free medium (a) were positive for focal adhesions and 29% of rSPARC-treated cells (b) were positive. Forty-eight percent of cells treated with either SPARC + anti-2.1 antibody (c) or with SPARC + anti-4.2 antibody (d) were positive for focal adhesions (i.e., cells having >3–5 plaques/cells). IR images of these cells are shown and focal adhesion plaques are denoted by arrowheads. Bar = 10 μm.

former, a cationic peptide from a non-Ca^{2+}-binding region of SPARC, has also been shown to inhibit [^{3}H]-thymidine incorporation into endothelial cell DNA at the same concentration of peptide (0.2 mM) that was effective in the focal adhesion assays [Funk and Sage, 1993], although it had no obvious effect on BAE cell spreading [Lane and Sage, 1990]. The peptide from the C-terminal Ca^{2+}-binding domain that was active in focal adhesion disassembly has been shown to block BAE cell spreading, alter stress fiber organization, and inhibit cell migration in response to basic fibroblast growth factor [Lane and Sage, 1990; Hasselaar and Sage, 1992]. However, peptide, 1.1 from the N-terminal, low affinity Ca^{2+}-binding domain, which prevents cell spreading, alters matrix gene expression, and blocks basic fibroblast growth factor-induced cell migration, was associated with only a partial loss of focal adhesions from BAE cells. Since cell rounding was observed at concentrations of peptide 1.1 $\geq$ 0.8 mM, and the peptide was used at 0.2 mM in the focal adhesion studies, it is possible that we might have observed greater decreases in focal adhesion-positive cells at higher concentrations. However, antibody to peptide 1.1, which effectively blocked SPARC-mediated cell rounding, had no effect on the ability of SPARC to induce focal adhesion disassembly [Lane and Sage, 1990] (and data not shown). In contrast, antibodies to peptides 2.1 and 4.2 blocked the dissociative effects of SPARC on adhesion plaques. These data indicate that peptides 2.1 and 4.2 contain sequences that induce focal adhesion disassembly and that either sequence alone is sufficient

to induce maximal loss of focal adhesions. Recent data show that these two peptides, but not peptide 1.1, can compete for the binding of SPARC to endothelial cells, and membrane proteins have been identified that are potential receptors for the EF-hand sequence [Yost and Sage, 1993]. At this point, it is not clear whether sequences represented by peptides 2.1 and 4.2 are recognized by the same or distinct receptors. The native molecule is folded such that the sequences represented by peptides 2.1 and 4.2 are in close proximity and, thus, might form a single binding pocket [Yost and Sage, 1993; Lane and Sage, 1994]. Nonetheless, these data support the idea that SPARC causes dissociation of focal adhesion structures through the triggering of intracellular responses resulting from its binding to cell surface receptors. It will be interesting to correlate specific receptor-ligand interactions with the biological activities of SPARC.

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