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# The Counteradhesive Protein SPARC Regulates an Endothelial Paracellular Pathway through Protein Tyrosine Phosphorylation

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**SPARC (Secreted Protein Acidic and Rich in Cysteine) regulates the transendothelial flux of macromolecules through a paracellular pathway. We now have demonstrated that SPARC-induced increments in albumin flux across postconfluent endothelial cell (EC) monolayers are mediated, in part, through protein tyrosine phosphorylation. SPARC increased tyrosine phosphorylation of EC proteins up to 12-fold within 1 h. The phosphotyrosine-containing proteins were immunolocalized to the intercellular boundaries. Two substrates for SPARC-induced tyrosine phosphorylation were identified as β-catenin and paxillin. Inhibition of tyrosine kinases with herbimycin A or genistein reversed the barrier dysfunction induced by SPARC by 71% and 49%, respectively. Herbimycin A also protected against SPARC-induced intercellular gap formation. In contrast, inhibition of tyrosine phosphatases with sodium orthovanadate or phenylarsine oxide enhanced the loss of barrier function associated with SPARC treatment by 120% and 88%, respectively. These data indicate that SPARC influences EC-EC interactions through a tyrosine phosphorylation-dependent signaling pathway.** 

SPARC (Secreted Protein Acidic and Rich in Cysteine), also known as osteonectin and BM-40, is a counteradhesive glycoprotein that limits the spreading of cells *in vitro* (1, 2). In endothelial cells (EC), it promotes the dissolution of focal adhesions (FA) and the partial detachment of cells from substrata *in vitro*, effects that promote a rounded cellular morphology (1-3). SPARC is secreted constitutively by several types of cultured cells including EC (4), and is expressed in tissues undergoing remodeling or morphogenesis *in vivo* (5). Increased expression of SPARC can be induced by injurious stimuli (4, 6) and, in EC, is characteristic of angiogenesis (7) and wound healing (8). Recently, we demonstrated that SPARC induces intercellular gap formation in postconfluent vascular EC monolayers coincident with increments in transendothelial albumin flux (9). Exposure of EC to SPARC was associated with the reorganization of actin, and prior stabilization of F-actin with phallicidin protected against the loss of barrier function. These findings were indicative of a paracellular pathway, regulated in part by the actin cytoskeleton, for the movement of macromolecules across the endothelium.

In EC, F-actin is arranged into both central transcytoplasmic cables and a peripheral band (10). These microfilaments are linked to two types of adherens junctions, FA (11, 12) and the zonula adherens (ZA) (13-15). The cytoplasmic filaments terminate within the FA and the subcortical filaments interdigitate with the ZA. Since, the ZA mechanically couples the peripheral actin cytoskeleton to the surface receptors that mediate homophilic cell-cell adhesion, it appears strategically located for regulation of the paracellular pathway (13-15). Although the signal transduction pathways that regulate the state of assembly of these adherens junctions are incompletely understood, tyrosine phosphorylation is known to modify target proteins within both structures. Tyrosine phosphorylation of both the FA (11, 12) and ZA (13-15) is associated with changes in the state of adherens junction assembly and increased vascular permeability (16, 17).

Although SPARC exhibits a specific binding to bovine aortic EC (18), neither the SPARC receptor/binding protein nor an intracellular effector mechanism has been defined. In the present report, we offer evidence that SPARC induces tyrosine phosphorylation of EC proteins. Two of these phosphotyrosine-containing proteins have been identified as the ZA component, β-catenin (13-15), and the FA component, paxillin (11-12). Moreover, tyrosine phosphorylation appears to mediate, at least in part, the formation of intercellular gaps induced by SPARC that are thought

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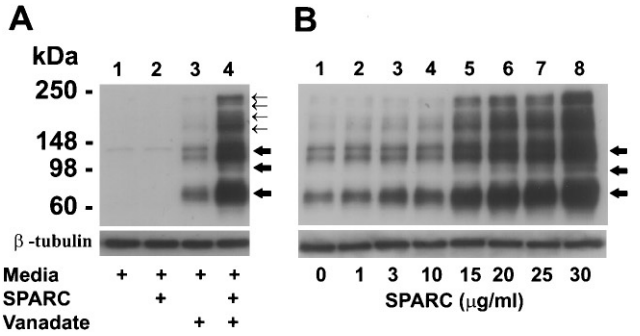


**FIG. 1.** Dose-dependent effect of SPARC on tyrosine phosphorylation of EC proteins. EC exposed to SPARC or to media alone were resolved by SDS-PAGE, transferred to PVDF membranes, and probed with an anti-phosphotyrosine (4G10) antibody. As confirmation of equivalent protein loading, each blot was stripped and reprobed with anti-$\beta$ tubulin antibody. Selected phosphotyrosine-containing bands increased in the presence of SPARC and vanadate are indicated by arrows on the right. These blots are representative of 4 experiments. (A) Phosphotyrosine-containing proteins in extracts of EC exposed to SPARC (20 $\mu$g/ml, 1 h) and to medium, in the presence and absence of vanadate (250 $\mu$M). Mr of protein standards in kDa are shown on the left. (B) Phosphotyrosine-containing proteins in EC extracts obtained from monolayers exposed for 1 h to increasing concentrations of SPARC in the presence of vanadate (250 $\mu$M).

to provide a paracellular pathway for the extravasation of macromolecules.

## METHODS

*EC Tissue culture.* Bovine pulmonary EC were obtained from American Tissue Culture Collection (Rockville, MD), and maintained as previously described (9, 19).

*Immunoblotting for EC phosphotyrosine.* Postconfluent EC monolayers were exposed for various times to increasing concentrations of purified SPARC (generously provided by Dr. E. Helene Sage, University of Washington, Seattle, WA) or to media alone, in the presence or absence of 250 $\mu$M sodium orthovanadate (vanadate) (Sigma, St. Louis, MO). EC were lysed with ice-cold lysis buffer and processed for immunoblotting with an anti-phosphotyrosine monoclonal antibody (0.75 $\mu$g/ml) (4G10, Upstate Biotechnology Inc. (UBI), Lake Placid, NY) as previously described (19). For confirmation of equivalent protein loading, specific blots were stripped with 100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl, pH 6.7, washed, blocked, and reprobed with 0.5 $\mu$g/ml of a anti-$\beta$-tubulin monoclonal antibody (Boehringer-Mannheim, Indianapolis, IN). The blots were then incubated with HRP-conjugated anti-mouse IgG (Transduction Laboratories, Lexington, KY), washed and developed with ECL. Autoradiographs were scanned by laser densitometry (Molecular Dynamics, Sunnyvale, CA).

*Assay of transendothelial albumin flux.* Transendothelial $^{14}$C-bovine serum albumin (BSA) flux expressed as pmole/h was assayed as previously described (9, 19). Briefly, gelatin-impregnated polycarbonate filters (Nucleopore, Inc., Pleasanton, CA) mounted in chemotaxis chambers (ADAPS, Inc., Dedham, MA), were inserted into wells of 24-well plates. Each upper compartment was seeded with 2 x $10^5$ EC and cultured for 72 h. Only monolayers retaining $\geq$97% of the $^{14}$C-BSA tracer were studied. EC monolayers were exposed to SPARC-enriched medium or to medium alone, in the presence or absence of either protein tyrosine kinase (PTK) or protein tyrosine phosphatase (PTP) inhibitors. In these experiments, monolayers were pretreated with genistein (185 $\mu$M), vanadate (2.5 $\mu$M), or PAO (0.1 $\mu$M) (Sigma), 0.5 h prior to and throughout the exposure to SPARC or media. Herbimycin A (1.0 $\mu$M) (Sigma) was introduced 16 h prior to and throughout the SPARC exposure.

*F-Actin epifluorescence microscopy and immunolocalization of phosphotyrosines.* EC grown to confluence on polycarbonate membrane filters were exposed for 6 h to SPARC (15 $\mu$g/ml, 0.45 $\mu$M) or to media alone. Selected cultures were incubated with herbimycin A (1.0 $\mu$M) for 16 h prior to and throughout the 6 h interval. The EC monolayers were fixed, stained with fluorescein-phalloidin (1.65 x

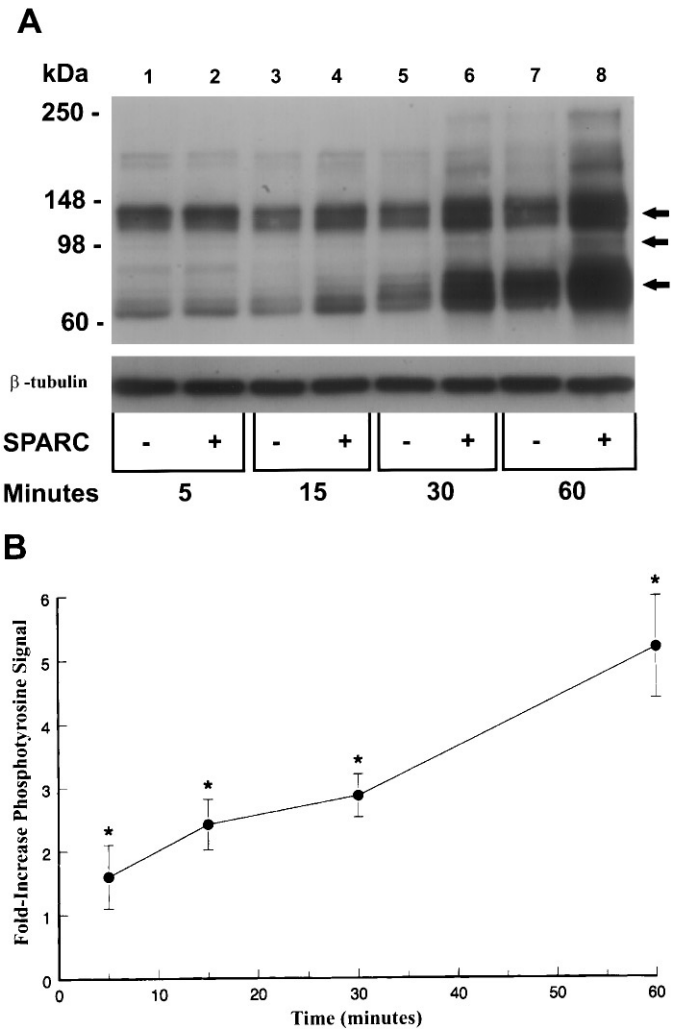


**FIG. 2.** Temporal effect of SPARC on protein tyrosine phosphorylation in EC. (A) EC exposed to SPARC (20 $\mu$g/ml) (+) or media alone (-) for increasing times in the presence of vanadate (250 $\mu$M) were processed for phosphotyrosine immunoblotting. Each blot was stripped and reprobed with anti-$\beta$ tubulin antibody. Mr of protein standards in kDa are shown on the left. Phosphotyrosine-containing bands increased in the presence of SPARC and vanadate are indicated by arrows on the right. This blot is representative of 4 experiments. (B) Time profile of SPARC-induced protein tyrosine phosphorylation expressed as mean densitometry units of the SPARC + vanadate lane $\div$ mean densitometry units of the lane with vanadate alone. Each data point represents the mean ($\pm$SE) of 3 experiments. *Significantly increased compared to simultaneous control at $p<0.05$.

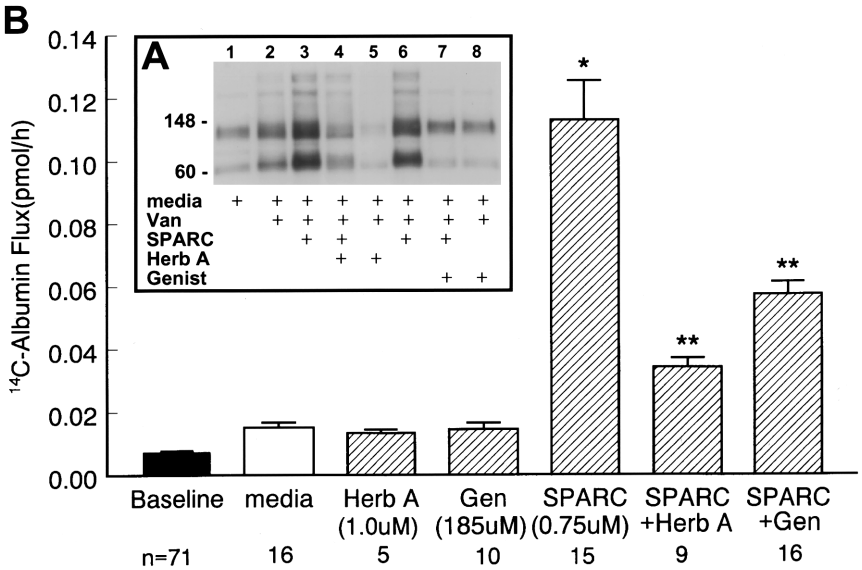


**FIG. 3.** Effect of PTK inhibition on SPARC-induced endothelial barrier dysfunction. (A) EC exposed to media, vanadate (250 $\mu$M), or SPARC (20 $\mu$g/ml) + vanadate (250 $\mu$M) in the presence or absence of either herbimycin A (1.0 $\mu$M) or genistein (185 $\mu$M) were processed for phosphotyrosine immunoblotting. This blot is representative of 3 experiments. (B) Vertical bars represent mean (±SE) transendothelial $^{14}$C-BSA flux in pmol/h immediately after 6 h exposure to SPARC (25 $\mu$g/ml), genistein (185 $\mu$M), herbimycin A (1.0 $\mu$M), SPARC + genistein, SPARC + herbimycin A, and the medium control. The pretreatment baseline is shown by the closed bar. *n* indicates the number of monolayers studied. *Significantly increased compared to the media control at $p<0.05$. **Significantly decreased compared to treatment with SPARC alone at $p<0.05$.

$10^{-7}$ M) (Molecular Probes, Inc.; Eugene, OR) and photographed for epifluorescence as previously described (9, 19). In other experiments, EC cultured to confluence on filters were probed for phosphotyrosine-containing proteins with a fluorescein isothiocyanate (FITC)-conjugated antiphosphotyrosine antibody (UBI) (5 $\mu$g/ml in PBS containing 1% BSA) as previously described (19).

*Immunoprecipitation of phosphotyrosine-containing proteins.* EC lysates were pre-cleared with anti-murine IgG cross-linked to agarose, incubated overnight with a murine monoclonal antibody raised against either $\beta$-catenin or paxillin (Transduction Laboratories) at 4°C, and the resultant immune complexes immobilized with anti-murine IgG cross-linked to agarose (Sigma). The immune complexes were pelleted by centrifugation, washed, boiled for 5 min in sample buffer, and again centrifuged. The supernates were processed for immunoblotting with anti-phosphotyrosine (4G10) antibody as described above. As a control for differences in loading of the immunoprecipitated proteins, blots were stripped as described above, washed, blocked and reprobed with the immunoprecipitating antibody.

*Statistical methods.* Analysis of variance (ANOVA) was used to compare the mean responses among experimental and control groups for all experiments. The Scheffe F-test was used to determine between which groups significant differences existed. A *p*-value of $<0.05$ was considered significant.

## RESULTS

*SPARC induces tyrosine phosphorylation of EC proteins.* Exposure of EC to a range of SPARC concentrations for varying time intervals demonstrated increases in protein tyrosine phosphorylation that could not be reproduced consistently (data not shown). However, in the presence of the PTP inhibitor, vanadate, SPARC treatment of EC for 1 h was clearly associated with tyrosine phosphorylation of a number of proteins (Fig. 1A, lane 4). Concentrations of SPARC as low as 3 $\mu$g/ml increased the phosphotyrosine signal in comparison to the effect seen in control EC (Fig. 1B, lane 3); at 30 $\mu$g/ml, SPARC increased the phosphotyrosine signal ≈12-fold (Fig. 1B, lane 8). At a fixed concentration of SPARC (20 $\mu$g/ml, 0.6 $\mu$M), exposure times as brief as 15 min were consistently associated with a >2-fold increase in phosphotyrosine-containing proteins (Fig. 2A, lanes 3 and 4). In several experiments, an ≈2-fold increase in tyrosine phosphorylation was evident as early as 5 min (Fig. 2B). In the presence of SPARC, phosphotyrosine-containing bands that migrated with apparent Mr of $140x10^3$-$120x10^3$, $95x10^3$ and $68x10^3$ (indicated by bold arrows in Figs. 1A,B and Fig. 2) were all increased ≈6-fold (Figs. 1A, lane 4; 1B, lane 6). Additional phosphotyrosine-containing proteins with apparent Mr of $240x10^3$, $220x10^3$, $185x10^3$, and $165x10^3$ were also apparent (Fig. 1). To investigate the specificity of this SPARC effect, equimolar concentrations (0.6 $\mu$M) of the adhesive proteins collagen I, fibronectin and laminin were each studied. Collagen I and laminin did not increase tyrosine phosphorylation of EC proteins under the same conditions as did SPARC (data not shown). Fibronectin induced a ≈1.5-fold increase in tyrosine phosphorylation of EC proteins, yet failed to augment $^{14}$C-BSA flux across EC monolayers (data not shown). These findings demonstrate the selective ability of SPARC to influence both

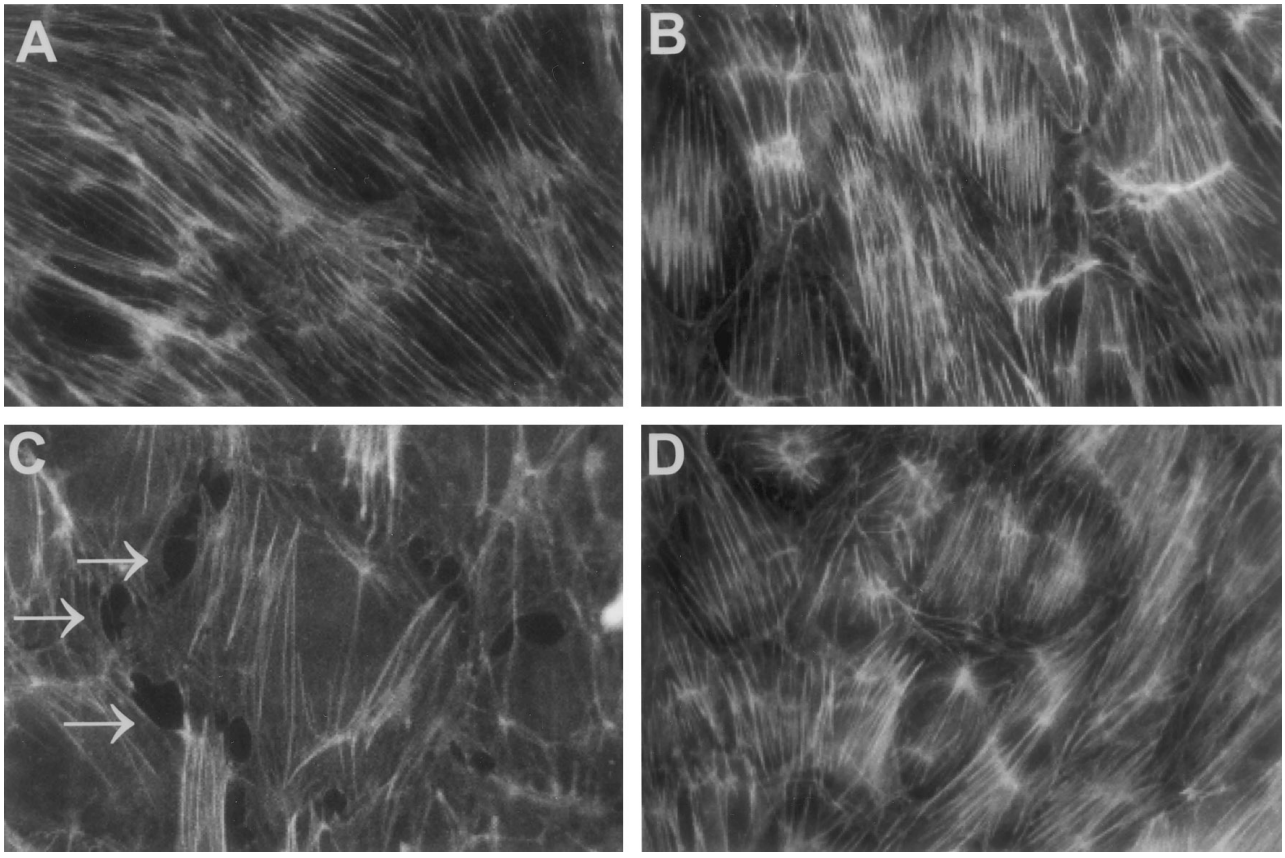


**FIG. 4.** Effect of PTK inhibition on intercellular gap formation between EC exposed to exogenous SPARC. EC exposed to media or SPARC (15 $\mu$g/ml) in the presence or absence of herbimycin A (1.0 $\mu$M) were stained with fluorescein-phalloidin, and analyzed by epifluorescence microscopy. (A) medium control, (B) herbimycin A control, (C) SPARC, (D) herbimycin A + SPARC. Arrows in C indicate intercellular gaps. X 750.

protein tyrosine phosphorylation and endothelial barrier function.

*PTK inhibition partially abrogates the changes in endothelial barrier function induced by SPARC.* PTK inhibition with either herbimycin A or genistein protected against SPARC-induced increments in transendothelial $^{14}$C-BSA flux (Fig. 3B). The mean ( $\pm$ SE) pretreatment baseline barrier function was 0.007 $\pm$ 0.000 pmol/h ($n$ = 71), and there were no significant differences among the experimental groups. The mean ( $\pm$ SE) $^{14}$C-BSA flux across naked filters without EC monolayers was 0.229 $\pm$ 0.020 pmol/h ($n$=5). $^{14}$C-BSA flux across EC monolayers treated with either herbimycin A or genistein alone was not different from flux across the media controls. An exposure to SPARC (25 $\mu$g/ml) for 6 h, significantly increased transendothelial $^{14}$C-BSA flux. Pretreatment of monolayers with either herbimycin A or genistein protected against the SPARC-induced increment by 71% and 49%, respectively. In addition, PTK inhibition with either herbimycin A or genistein decreased the protein tyrosine phosphorylation that was observed in the presence of the SPARC stimulus (Fig. 3A). Herbimycin A decreased both the ≈140-120kDa and ≈68kDa bands by 87%, whereas genistein decreased the two bands by 68% and 92%, respectively. In addition, herbimycin A and genistein each decreased the ≈95kDa band by 97%. Therefore, two structurally and functionally dissimilar PTK inhibitors each diminished the SPARC effect.

*PTK inhibition protects against the intercellular gap formation promoted by SPARC in cultured EC.* EC monolayers exposed to SPARC (15 $\mu$g/ml,) $\pm$ herbimycin A, were stained with fluorescein-phalloidin, an F-actin-specific reagent. By fluorescence microscopy, monolayers incubated with herbimycin A or media alone exhibited continuous transcytoplasmic actin filaments and cell-to-cell apposition without intercellular gaps (Fig. 4A,B). After exposure to SPARC for 6 h, isolated ellipsoid disruptions within the F-actin lattice occurred predominantly at the cell-cell interface (Fig. 4C). In EC monolayers preincubated with herbimycin A for 16 h prior to and throughout the 6 h exposure to SPARC, no intercellular gaps were evident (Fig. 4D). Therefore, inhibition of PTKs appears to block the for-

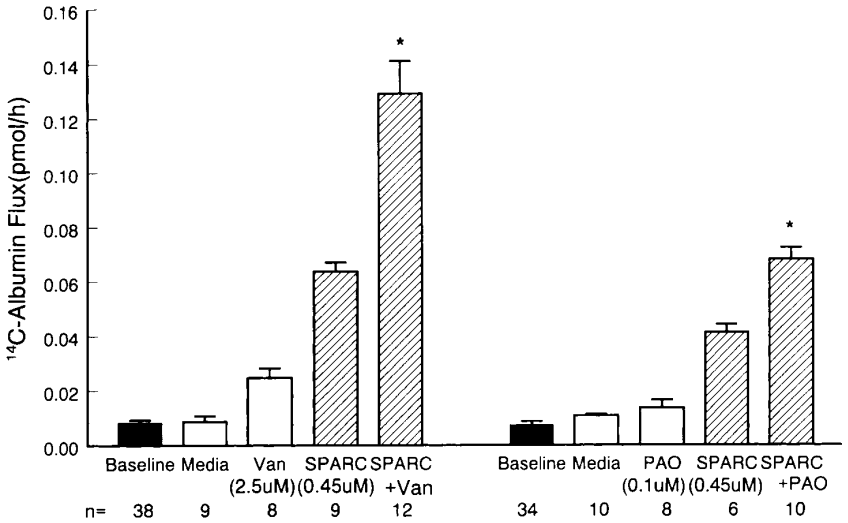


**FIG. 5.** Effect of PTPinhibition on SPARC-induced changes in endothelial barrier function. Vertical bars represent mean (±SE) transendothelial $^{14}$C-BSA flux in pmol/h immediately following 6 h exposures to SPARC (15 $\mu$g/ml), vanadate (2.5 $\mu$M), PAO (0.1 $\mu$M), SPARC + vanadate, SPARC + PAO, and the media controls. The pretreatment baselines are shown by the closed bars. *n* indicates the number of monolayers studied. *Significantly increased compared to treatment with SPARC alone, at $p<0.05$.

mation of gaps between confluent EC associated with SPARC exposure.

*PTP inhibition enhances the effect of SPARC on endothelial barrier function.* Whereas inhibition of PTK activity protected against the loss of barrier function observed in the presence of SPARC (Fig. 3), inhibition of PTP activity enhanced the effect (Fig. 5). The mean ( ± SE) pretreatment baseline barrier function was 0.008 ± 0.000 pmol/h ($n=72$). Pretreatment of monolayers with either vanadate or PAO alone did not significantly increase $^{14}$C-BSA flux in comparison to the media controls. Pretreatment with either of the two PTP inhibitors enhanced the SPARC effect. These data provide additional evidence that the influence of SPARC on endothelial barrier function is mediated through tyrosine phosphorylation of one or more EC proteins.

*Phosphotyrosine immunolocalization to intercellular boundaries of EC after exposure to SPARC.* EC monolayers exposed to SPARC or media alone were probed with a FITC-conjugated anti-phosphotyrosine antibody and developed with epifluorescence microscopy (Fig. 6). Only EC incubated with SPARC displayed a fluorescence signal that was predominantly restricted to the intercellular boundaries (Figs. 6B,D), whereas the cells provided with media alone lacked this pattern of immunofluorescence (Figs. 6A,C). These studies indicate that the increase in phosphotyrosine-containing proteins in SPARC-exposed EC appears to be localized to the cell-cell boundaries.

*Identification of the ≈95kDa and ≈68kDa tyrosine-phosphorylated proteins in EC exposed to SPARC.* EC treated with SPARC (20 $\mu$g/ml, 1h) and immunoscreened for phosphoproteins revealed that $\beta$-catenin and paxillin comigrated with the ≈95kDa and ≈68kDa bands, respectively (data not shown). To confirm the identification of these phosphoproteins, lysates from EC were immunoprecipitated with anti-paxillin and anti-$\beta$-catenin antibodies. These experiments demonstrated that SPARC increased the tyrosine phosphorylation of paxillin (Fig. 7, lane 2) and $\beta$-catenin (Fig. 7, lane 4) relative to controls, ≈2-fold and ≈4-fold, respectively.

## DISCUSSION

In this study, we have demonstrated that SPARC influences transendothelial flux of macromolecules through a paracellular pathway, the functional state of which is regulated through protein tyrosine phosphorylation. The dose (≥3 $\mu$g/ml) and time (≥5 min) requirements for tyrosine phosphorylation were compatible with those we previously reported for changes in endothelial barrier function induced by SPARC (9). PTK inhibitors in concentrations that blocked tyrosine phosphorylation of EC proteins in response to SPARC protected against SPARC-induced opening of the paracellular pathway and increments in transendothelial albumin flux. In contrast, PTP inhibition enhanced the barrier dysfunction associated with SPARC exposure. Finally, two phosphotyrosine-containing proteins were identified as the adherens junction proteins, $\beta$-catenin (13-15) and paxillin (11, 12). These combined data support our proposal that SPARC regulates EC barrier function, in part, through tyrosine phosphorylation of one or more adherens junction proteins.

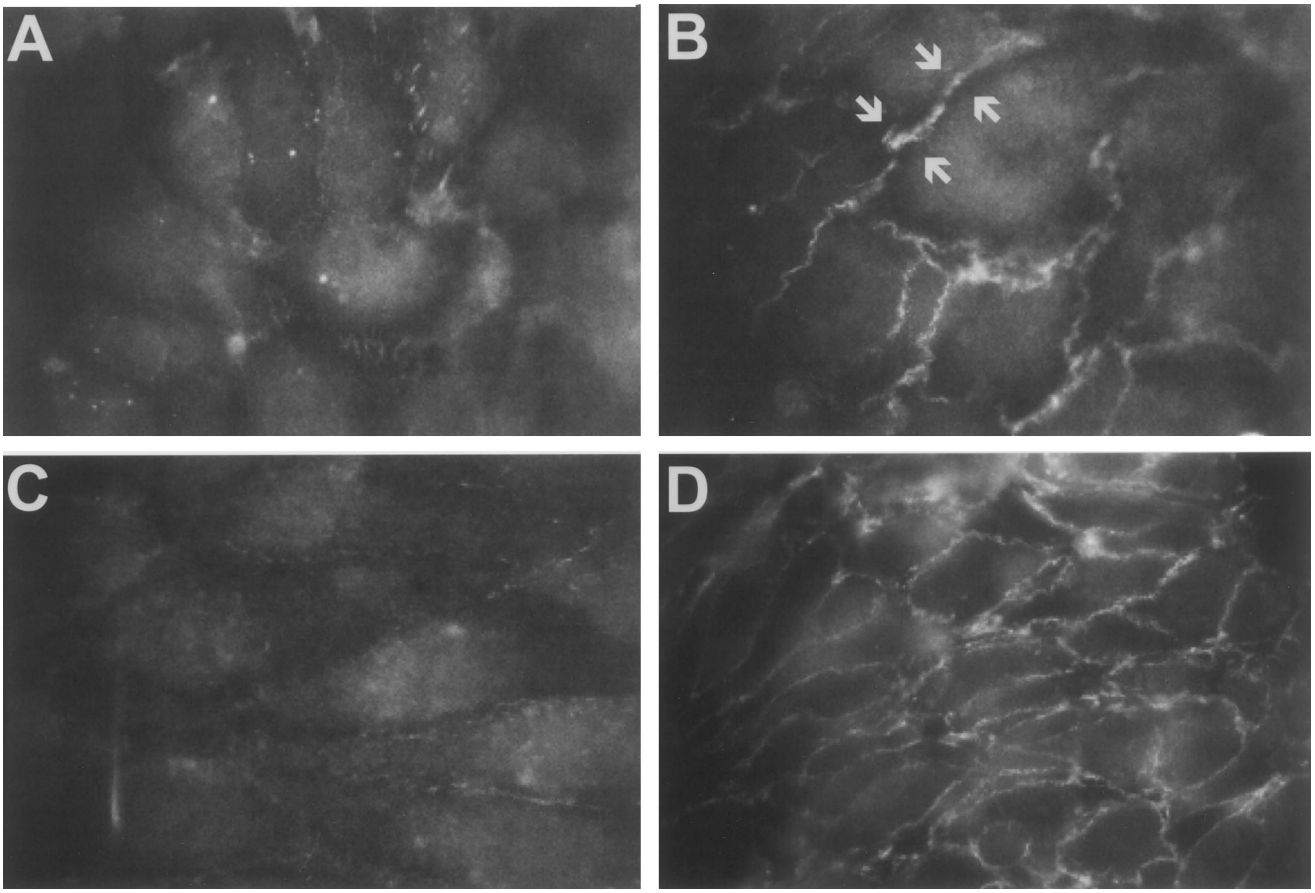


**FIG. 6.** Immunolocalization of phosphotyrosine-containing proteins in EC exposed to SPARC. EC exposed to SPARC (20 $\mu$g/ml) or media were incubated with a FITC-conjugated antiphosphotyrosine antibody and analyzed by epifluorescence microscopy. (A) Medium control for 5 min, (B) SPARC for 5 min, (C) medium control for 1 h, (D) SPARC for 1 h. Arrows in B point to phosphotyrosine signal concentrated at intercellular boundaries of EC exposed to SPARC. X750.

The state of protein tyrosine phosphorylation is central to regulating cell-cell adhesion (13-15). More specifically, maintenance of endothelial barrier integrity is influenced by tyrosine phosphorylation of adherens junction proteins (16, 17). PTPs are thought to play a crucial role in controlling the state of adherens junction assembly (20-26). The activity of receptor PTPs increases as cell progress to confluence, *in vitro* (20, 21). In EC, the activation of the receptor PTP, HPTP$\beta$, increases 12-fold during monolayer formation (20). Two receptor PTPs, PTP$\mu$ (22) and $PTP_k$ (23), each localize to cell-cell adherens junctions, directly bind to ZA component proteins and participate in homophilic adhesion. Interestingly, the receptor PTPs, $PTP_k$ (23) and LAR-PTP (24), and the nonreceptor PTP, PTP1B-like phosphatase (25), each bind to and dephosphorylate $\beta$-catenin. The ability of both receptor and nonreceptor PTPs to associate with and dephosphorylate specific ZA components provides a mechanism(s) through which tyrosine phosphorylation within the ZA and consequently homophilic adhesion can be regulated. That PTP inhibition unmasks or enhances tyrosine phosphorylation in the presence of SPARC as well as the downstream events of intercellular gap formation and loss of barrier function are compatible with these findings.

One substrate for SPARC-induced tyrosine phosphorylation was identified as paxillin, a highly conserved ≈68kDa protein that appears to be restricted to FAs (11, 12, 27). Multiple PTKs have been shown to bind to and phosphorylate paxillin on tyrosine including src and FAK (11, 12, 27). Tyrosine phosphorylation of paxillin is associated with changes in the state of FA assembly (11, 12, 27). Under most conditions, including those that activate the rhoA signaling pathway, tyrosine phosphorylation of paxillin is associated with stress fiber formation and FA assembly (28, 29). However, src transformation induces tyrosine phosphorylation of paxillin and FA disassembly (12). Whether SPARC-induced disassembly of adherens junctions could be mediated, in part, through src PTK activity, is unclear.

Another identified substrate for SPARC-induced tyrosine phosphorylation was the ≈95kDa structural

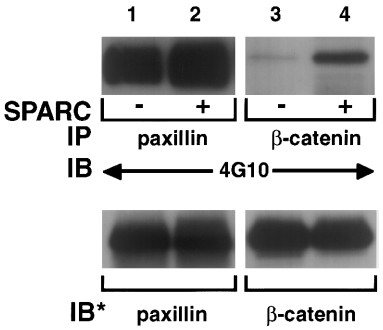


**FIG. 7.** Identification of the tyrosine-phosphorylated proteins, β-catenin and paxillin, in EC exposed to SPARC. EC incubated with SPARC (20 μg/ml) or media in the presence of vanadate (250 μM) were immunoprecipitated with either anti-β-catenin or anti-paxillin antibody and processed for immunoblotting with an antiphosphotyrosine (4G10) antibody. Blots were stripped and reprobed with the immunoprecipitating antibodies, i.e., anti-β-catenin or anti-paxillin. IP = immunoprecipitate, IB = immunoblot, IB* = immunoblot after stripping. These blots are representative of 3 experiments.

and regulatory protein, β-catenin (13-15). β-catenin binds directly to the cytoplasmic tail of cadherin and through its interaction with α-catenin, links the cadherins to the actin cytoskeleton (13-15). Tyrosine phosphorylation of β-catenin promotes ZA disassembly, reduces homophilic intercellular adhesion, and promotes a rounded cell morphology (13-15,30-33). Tyrosine phosphorylation of β-catenin and disruption of cell-cell adhesion have been shown to be induced by both src and ras transformation, and by activation of selected mitogenic growth factor receptors (30-33,34,35). In several studies, PTK inhibition that blocked increased tyrosine phosphorylation of β-catenin restored ZA integrity (30,32,33,36). Here, we have demonstrated that PTK inhibition blocked SPARC-induced increases in tyrosine phosphorylation of a ≈95kDa band that comigrated with β-catenin (Fig. 3A), and protected against SPARC-induced intercellular gap formation (Fig. 4). Thus, SPARC may regulate endothelial barrier function, in part, through tyrosine phosphorylation of the ZA component, β-catenin.

The participation of the ZA in cell-cell adhesion depends on its linkage to the actin cytoskeleton and the state of tyrosine phosphorylation of its protein components (13-15). Certain features of EC barrier dysfunction promoted by SPARC indicate involvement of the ZA. First, SPARC induces intercellular gaps at the cell-cell boundaries (9) where the ZA is located. Second, SPARC induces actin reorganization in EC, and prior F-actin stabilization with phallicidin protects against the barrier dysfunction seen in the presence of SPARC (9). Third, this loss of barrier function is tyrosine phosphorylation-dependent (Figs 3, 4, and 5) and the increased expression of phosphotyrosine-containing proteins is concentrated at the intercellular boundaries (Fig. 6). Finally, SPARC induces tyrosine phosphorylation of β-catenin (Fig. 7), a key signaling and structural protein within the ZA. Collectively, these data indicate that the counteradhesive protein SPARC perturbs cell-cell as well as cell-matrix interactions.

The regulation of the transendothelial paracellular pathway through protein tyrosine phosphorylation may also involve other intercellular junctions. Changes in ZA integrity appear to influence the competence of another specialized intercellular junction, the zonula occludens or tight junction (37). Other molecules that may influence EC-EC adhesion through tyrosine phosphorylation include the platelet/endothelial cell adhesion molecule-1 (CD31) (38, 39) as well as the α2β1 and α5β1 integrins (40). It is possible that any changes in ZA integrity induced by SPARC cooperatively influence these other intercellular interactions. SPARC induces tyrosine phosphorylation of proteins associated with both cell-matrix and cell-cell adherens junctions (Fig. 7) Whether such modifications in either adherens junction can influence the state of assembly of the other, is unclear.

SPARC has been identified as the prototypic counteradhesive protein that clearly disrupts cell-matrix interactions (5) and is operative over a wide range of complex intercellular interactions. In this study, we have presented evidence for the modulation of EC-EC interactions by SPARC, in part, through a pathway dependent on the tyrosine phosphorylation of one or more proteins that include β-catenin and/or paxillin.

## ACKNOWLEDGMENTS

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