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Absence of SPARC in Lens Epithelial Cells Results in Altered Adhesion and Extracellular Matrix Production In Vitro

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Abstract The matricellular protein SPARC (also known as osteonectin and BM-40) is expressed abundantly in lens epithelium. That SPARC-null mice exhibit early cataractogenesis, indicates a role for SPARC in the maintenance of lens transparency. Comparison of cultured wild-type and SPARC-null lens epithelial cells revealed significant changes in adhesion to different substrates. SPARC-null lens cells displayed enhanced attachment and spreading, focal adhesion formation, and resistance to trypsin detachment in comparison to wild-type cells. In the absence of SPARC, there was increased deposition of the ECM protein laminin-1 (LN-1). Proteins associated with focal adhesions were increased in SPARC-null versus wild-type lens cells: levels of α6-integrin heterodimers, talin, and paxillin phosphorylated on tyrosine were enhanced significantly, as was the association of β1-integrin with talin and paxillin. Restoration of the wild-type phenotype in SPARC-null cultures was accomplished through genetic rescue by stable transfection of SPARC cDNA. Our findings indicate that SPARC is counter-adhesive for murine lens epithelial cells and demonstrate that multiple factors contribute to this activity. We also identify SPARC as a modulator of LN-1 secretion and deposition by these cells, an activity important in epithelial cell-ECM interactions in the ocular lens. J. Cell. Biochem. 97: 423–432, 2006.

Key words: SPARC; extracellular matrix; lens epithelial cells; adhesion; laminin-1; osteonectin

SPARC (secreted protein acidic and rich in cysteine), also known as osteonectin and BM-40, is a calcium-binding glycoprotein belonging to a group of matricellular proteins that include thrombospondin-1 and 2, hevin/SC-1, osteopontin, tenascin C and X, and Cyr61 [Bornstein and Sage, 2002]. Matricellular proteins have been shown to interact with integrin receptors, growth factors, and structural components of the extracellular matrix (ECM). Acting at the interface between the cell surface and the ECM, matricellular proteins affect cellular processes such as adhesion, migration, proliferation, and differentiation [Brekken and Sage, 2001; Bornstein and Sage, 2002].

SPARC has been implicated in angiogenesis, tumor invasion, cataractogenesis, and wound healing. It has been shown that SPARC has counter-adhesive activity on non-lens cells in vitro, for example, endothelial cells [Lane and Sage, 1990; Motamed and Sage, 1998], glioma cells [Rempel et al., 2001], fibroblasts [Bradshaw et al., 1999], smooth muscle cells [Sage et al., 1989], and transfected F9 tetracarcinoma cells [Everitt and Sage, 1992], in part by disruption of focal adhesion complexes and by prevention of cell spreading through unidentified mechanisms [Everitt and Sage, 1992; Murphy-Ullrich, 2001; Murphy-Ullrich et al., 2001]. Focal adhesions provide structural support between the ECM and the intracellular cytoskeleton as well as a conduit for specific intracellular signaling cascades in response to extracellular signals, for example, via tyrosine phosphorylation of intracellular proteins [Schwartz, 2001].

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The lens capsule, a specialized ECM, is composed mainly of collagen IV, laminin (LN)-1, perlecan, and nidogen, and is produced primarily by lens epithelial cells (LEC) [Arita et al., 1993]. Maintenance of lens transparency requires precisely coordinated interaction between the capsular ECM proteins and the basal surface of the LEC, a molecular recognition that regulates their migration, proliferation, adhesion, and differentiation [Zelenka, 2004]. SPARC is expressed abundantly in lens epithelium, and participates in the regulation of ECM protein production. SPARC-null mice display altered expression of capsular ECM proteins that have been implicated in adhesion [Yan et al., 2002, 2005b].

The objective of the present study was to understand how SPARC modulates LEC adhesion. Our approach included studies on focal adhesion formation and stability, ECM protein deposition/secretion, integrin heterodimer formation, and adhesion-related intracellular signaling events in these cells. SPARC was found to inhibit LEC adhesion by several potentially-related mechanisms that include diminishment of focal adhesions, inhibition of LN-1 deposition, downregulation of α6-integrin heterodimer formation, and reduction of tyrosine-phosphorylated paxillin.

MATERIALS AND METHODS

Cell Culture and Transfection

LEC were cultured from lenses of wild-type (WT) and SPARC-null (null) C57Bl6/129SVJ mice of 1–2 months of age as described previously [Yan et al., 2005a]. Spontaneously, immortalized LEC lines (WT/7803 and SP-KO/7803) were established in our laboratory and were used for experiments described below. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco-BRL, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (Gibco-BRL), 100 U/ml penicillin G, and 100 μg/ml streptomycin SO_4 at 37°C in a humidified atmosphere containing 5% CO_2. For long-term culture, cells were subcultured in 10% FBS/DMEM and were split at a ratio of 1:3. For rescue experiments, SPARC-null cells were transfected with SPARC-green fluorescent protein (GFP) cDNA or GFP cDNA (control) with a cytomegalovirus (CMV) promoter-driven expression vector, as described previously [Yan et al., 2005a]. GFP-expressing LEC are SPARC-negative cells, whereas SPARC-GFP-expressing LEC are SPARC-positive cells. Stable transfection was maintained in 10% FBS/DMEM with 500 μg/ml geneticin (Gibco-BRL).

Immunocytochemistry

Cells grown on glass coverslips in 10% FBS overnight were fixed with 3.7% formaldehyde in phosphate-buffered saline (PBS) for 10 min. Staining was performed with an anti-mouse SPARC IgG (10 μg/ml; R&D Systems Inc., Minneapolis, MN), anti-vinculin mouse IgG (10 μg/ml; Sigma, St. Louis, MO), or an anti-α6-integrin rat IgG (20 μg/ml; Chemicon, Temecula, CA), followed by a tetramethylrhodamine isothiocyanate (TRITC)- or fluorescein isothiocyanate (FITC)-conjugated secondary antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). Nuclei were stained with Hoechst dye 33258 (4 μg/ml; Invitrogen, Carlsbad, CA) for 2 min. Cells were photographed with a Leica fluorescence microscope (Leica Microsystems, Wetzlar, Germany).

Cell Attachment/Spreading

Cells were grown in 10% FBS/DMEM for 48 h, washed twice with Mg^{2+}/Ca^{2+}-free PBS, and incubated in serum-free DMEM overnight. Cells were detached with trypsin, neutralized with FBS/DMEM, washed three times with serum-free DMEM, and plated in 0.5% FBS/DMEM for the times indicated. Cells were photographed at indicated times with a Leica DM IL inverted microscope (Leica Microsystems). For quantitative analysis, cells were washed in PBS and were fixed for 30 min in 5% glutaraldehyde in PBS at RT. Following fixation, cells were stained for 30 min with 0.1% crystal violet and were destained by washing extensively with deionized water. The bound dye was solubilized with 4% sodium dodecyl sulfate (SDS) in PBS, and the absorbance at 560 nm was read on an $OPTI_{max}$ microplate reader (Molecular Devices, Sunnyvale, CA).

For assessment of the effect of ECM deposition on adhesion, equal numbers of GFP-transfected and SPARC-GFP-transfected LEC were plated on 6-well culture plates in 10% FBS/DMEM. After 8–10 h, the adherent cells were washed with PBS and were incubated in serum-free DMEM for 72 h. Subsequently, cells were removed from plates with repeated washes in Mg^{2+}/Ca^{2+}-free PBS, and each well was re-plated

with a total of $2x10^5$ SPARC-null LEC per well in 0.5% FBS/DMEM for the times indicated. Cells were photographed as described above. For quantification, a scoring system was used to characterize the progression of spreading within a cell population. Cells were counted and scored as follows: (a) spread, flat cells with low refractility; (b) non-spread, rounded cells with short processes indicating initial spreading; (c) round cells with no processes and high refractility. The numbers from each group were converted into a Rounding Index (RI) from randomly-selected fields by the formula: $RI = [1 \times a) + (2 \times b) + (3 \times c]/(a + b + c)$ where a, b, and c are the number of cells in each population [Lane and Sage, 1990]. All initially plated cell populations have an RI of 3, as all cells are rounded and have yet to extend processes. As a population attaches and spreads on a surface, the index approaches a value of 1, reflecting a completely spread culture. RI averages were calculated from three independent experiments and graphed as the average ± SE.

ECM Proteins in Lens Epithelial Cell Culture

For quantification of ECM deposition and/or secretion, cells were seeded into 6-well culture plates at 2×10^5 cells/well and were incubated in 10% FBS/DMEM overnight. Cells were washed with DMEM and were subsequently incubated with DMEM containing 0.1% bovine serum albumin (BSA) for 72 h. At the end of the incubation, the media were collected, centrifuged briefly at 4°C to remove cells and debris, and supplemented with 1× complete protease inhibitor cocktail containing inhibitors targeting chymotrypsin, thermolysin, papain, pronase, pancreatic extract, and trypsin (Roche Diagnostics, Mannheim, Germany). The cells in each well were removed from the plate by repeated washing with Mg^{2+}/Ca^{2+}-free PBS at 37°C. Detached cells were counted and were subsequently lysed with Mammalian Protein Extraction Reagent (M-PER; Pierce Biotechnology, Rockford, IL) containing complete protease inhibitor cocktail. Conditioned media (CM) corresponding to equal cell numbers were concentrated fivefold by YM-50 Centricon devices (Millipore, Bedford, MA). Proteins deposited on the plates were collected by scraping with a policeman into M-PER. Protein concentrations of cell lysates were determined by a bicinchoninic acid assay (Pierce Biotechnology). Cell lysate (10 μg) and concentrated CM from each treatment were fractioned by SDS–PAGE and subjected to Western blotting with polyclonal antibodies against LN-1 (2 μg/ml; Sigma) or FN (10 μg/ml; Sigma). The blot was stripped and reprobed with anti-GAPDH IgG (1 μg/ml; Ambion, Austin, TX). Control plates, treated identically but without cells, were processed as described above to evaluate growth media-associated protein deposition.

Flow Cytometry Analysis of α6 Integrin

LEC were incubated for 24 h in 0.5% FBS/DMEM. Cells were washed twice in PBS and were removed from plates with PBS containing 4% ethylenediaminetetraacetic acid (EDTA). Cells were centrifuged and re-suspended in wash buffer (PBS, 1% FBS, 0.01% sodium azide, 0.5 mM Mn^{2+}) and were stained with rat anti-α6 integrin (10 μg/ml; Chemicon), rat anti-β1-integrin (10 μg/ml; Chemicon), or an isotype rat IgG control (10 μg/ml; Chemicon). Cells were washed and incubated with (FITC)-conjugated anti-rat secondary antibody (Jackson ImmunoResearch Laboratories, Inc.). Flow analysis was performed with a FacsCaliber system (BD Biosciences, San Diego, CA).

Phosphotyrosine Analysis

Cells were grown in 10% FBS/DMEM for 48 h and were subsequently incubated in serum-free DMEM overnight. Cells were detached and suspended in DMEM or were plated at equal cell numbers ($2x10^6$ cells/60 mm dish) in serum-free media for the times indicated. Cells were collected in lysis buffer containing 1% NP-40, 50 mM Hepes, pH 7.2, 150 mM NaCl, 5 mM Na_3VO_4, 5 mM NaF, and 1× complete protease inhibitor cocktail. Cell lysates were resolved by SDS–PAGE on 4–12% reducing NuPAGE® Novex Bis-Tris (Bis (2-hydroxyethyl) imino-tris (hydroxymethyl) methane-HCl) pre-cast gels (Invitrogen). Tyrosine (Tyr)-phosphorylated proteins were detected with a mouse anti-phoshoTyr antibody (200 ng/ml; Santa Cruz Biotechnology, Santa Cruz, CA). Individual Tyr-phosphorylated proteins were identified with protein-specific antibodies: anti-paxillin IgG (25 ng/ml; BD Biosciences) and anti-talin IgG (Sigma).

Co-Immunoprecipitation

Lysates, prepared as described above, were pre-cleared for 1 h at 4°C with Sepharose-G (East Coast Biologics, Inc., North Berwick, ME)

and were subsequently immunoprecipitated overnight at 4°C with a hamster anti-β1-integrin IgG (1 μg β1 antibody/250 μg lysate; Santa Cruz Biotechnology). Immunoprecipitates were centrifuged and were washed three times with lysis buffer containing increasing concentrations of NaCl (150 mM, 450 mM, and 750 mM). After the final wash, immunoprecipitates were resolved by SDS–PAGE on 4–12% reducing, NuPAGE® Novex Bis-Tris pre-cast gels (Invitrogen). Association of talin with β1-integrin was detected with talin-specific antibodies as described above.

RESULTS

SPARC-Null LEC Exhibited Increased Adhesion In Vitro

Expression of SPARC was apparent in WT but not in null LEC in culture (Fig. 1A,B). We validated specificity of the effect of SPARC on LEC by genetic rescue. SPARC-null LEC were stably transfected with a vector expressing GFP or SPARC-GFP fusion protein driven by a CMV promoter. Expression of the fusion protein was verified by immunostaining and Western blotting (Fig. 1C–E). The expression levels of CMV-driven SPARC-GFP (~73 kD) in transfectants were comparable to the levels of SPARC via the endogenous promoter observed in WT LEC (43 kD) (Fig. 1E).

To test whether SPARC could mediate attachment/spreading of LEC in vitro, we plated LEC on tissue culture plates in low serum (Fig. 2). SPARC-null LEC showed enhanced attachment and spreading in 0.5% FBS/DMEM (Fig. 2A–C), in comparison to WT cells (Fig. 2D–F). After 1 h or 3 h incubation, quantification of cell spreading by crystal violet staining of cells indicated nearly a onefold increase in adhesion in SPARC-null over WT LEC (Fig. 2G). Although exhibiting slower adhesion, the WT LEC were eventually spread on the plates under these conditions (data not shown).

Alterations in adhesion led us to examine the formation of focal adhesion plaques in cultured LEC. SPARC-null LEC showed more broadly-distributed vinculin-containing focal adhesions, and a slower rate of trypsin-induced detachment, compared with WT LEC (data not shown).

Changes in Secreted ECM in SPARC-Null LEC

To examine the possibility that SPARC-mediated ECM deposition affects cell adhesion, we allowed GFP-transfected and SPARC-GFP-transfected LEC to deposit ECM on plates over a period of 72 h. LEC were subsequently removed with Ca^{2+}/Mg^{2+}-free PBS washes. Complete removal of cells was verified by nuclear staining with Hoechst dye 33258. Plates with LEC-deposited ECM were re-seeded with equal

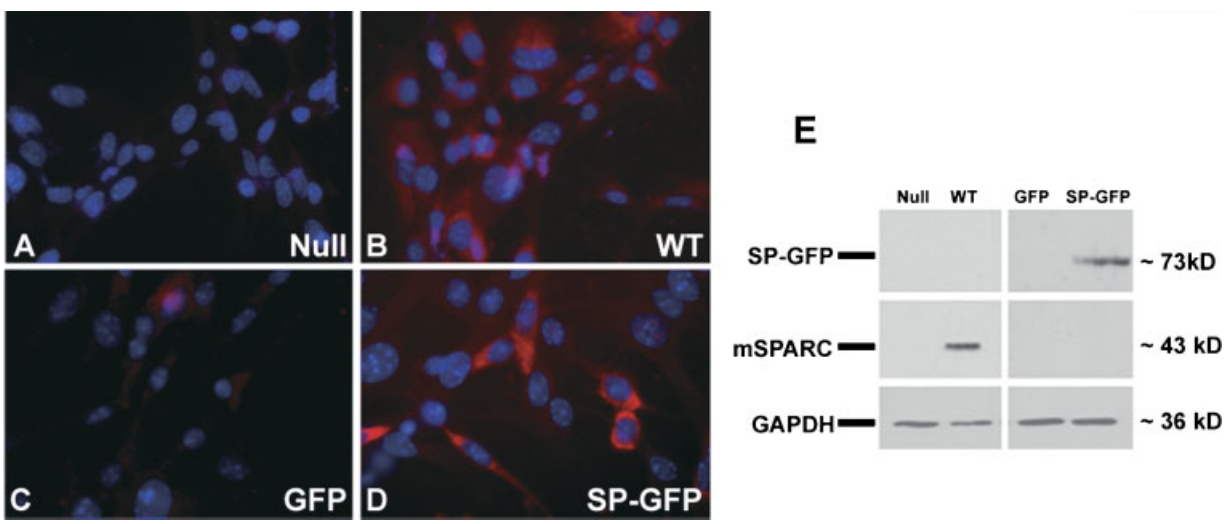

Fig. 1. SPARC expression in cultured LEC. LEC were cultured from the lenses of WT and SPARC-null mice 1–2 months of age. SPARC-null (Null) (**A**), WT (**B**), or SPARC-null cells transfected with GFP cDNA (**C**), or SPARC-GFP (SP-GFP) cDNA (**D**) were plated in 10% FBS/DMEM on glass coverslips for 24 h. Expression of SPARC was revealed by immunostaining of LEC with anti-SPARC IgG in both WT (B) and SP-GFP (D) transfected cells. **E**: Western blot for SPARC showed levels of SP-GFP (73 kD) that were comparable to SPARC in WT LEC (43 kD). GAPDH was used as an internal control. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

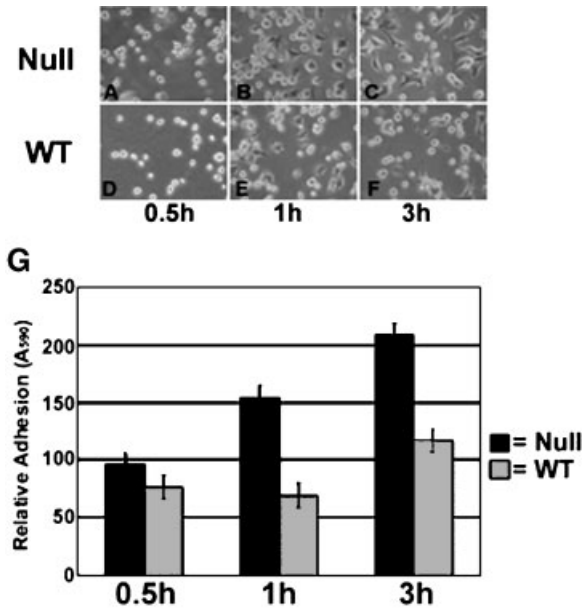

Fig. 2. SPARC-null LEC exhibit increased adhesion. SPARC-null (**A**–**C**) and WT (**D**–**F**) LEC were incubated in serum-free DMEM for 24 h, detached with trypsin, plated for the indicated times in 0.5% FBS/DMEM, and photographed under a phase contrast microscope. **G**: Cells were fixed with 5% glutaraldehyde and were stained with 0.1% crystal violet. Absorbance at 590 nm (A_{590}) was determined on an ELISA plate reader to assess relative adhesion. Data represent means ± standard deviation of three experiments.

numbers of SPARC-null LEC. SPARC-null LEC displayed increased adhesion on ECM deposited by LEC expressing only GFP (Fig 3A–C), compared to that of LEC that were transfected with SPARC-GFP (Fig. 3D–F). A Rounding Index was used to quantify cell spreading on each substrate composed of ECM proteins. Spreading of LEC was enhanced significantly at 1 h and 4 h time points ($P < 0.02$) on ECM proteins deposited by GFP-expressing cells alone relative to those expressing SPARC-GFP (Fig. 3G).

Data in Figure 3 indicated that the ECM proteins deposited by LEC with or without endogenous SPARC resulted in differential spreading. For quantitative analysis of the respective ECM proteins, Western blotting was performed on the ECM secreted or deposited by cultured LEC under serum-free conditions. Levels of LN-1 secreted and deposited by null cells were two times and four times the values of the corresponding WT cells, respectively (Fig. 4). Secreted and deposited FN levels were also increased from SPARC-null LEC relative to WT cells (data not shown). Equal protein loading and lack of cellular contamination were controlled in these samples by subsequent blotting for GAPDH. A similar observation was made when comparing stably transfected LEC. Increased secretion and deposition of LN-1 was seen in GFP relative to SPARC-GFP-transfected LEC (Fig. 4). These results demonstrate a SPARC-mediated control of LN-1 secretion and deposition by LEC. Changes in the secretion/deposition of this ECM protein, modulated by SPARC, contribute at least in part, to the counter-adhesive function of SPARC in LEC.

Integrin Activation and Intracellular Signaling in SPARC-Null LEC

Ligand binding to integrins is rapidly regulated by integrin activation. Given the substantial changes in LN-1 between WT and SPARC-null LEC, we focused on LN-associated integrin dimerization. Flow cytometry was performed with an antibody reactive against the alpha-6 integrin subunit when dimerized with a β-subunit, for example, α6β1 or α6β4, integrin heterodimers that have been shown to act as LN-1 cellular receptors [Mercurio et al., 2001]. SPARC-null LEC displayed a onefold increase in α6-integrin heterodimer expression in comparison to WT LEC after an incubation of 12 h in 0.5% FBS/DMEM (Fig. 5A). Consistently, SPARC-null LEC grown on glass coverslips for 12 h in 0.5% FBS/DMEM exhibited enhanced staining, relative to that of WT, of α6-integrin heterodimers by immunocytochemistry (Fig. 5B). Subsequent double staining of α6-integrin and vinculin revealed that the α6-integrin heterodimers were distributed along the periphery and over the center of the SPARC-null LEC, and were co-localized with focal adhesion complexes (data not shown). This result indicates that SPARC-null LEC have increased α6β1 and/or α6β4 heterodimers that appear coincident with focal adhesions. Since LN-1 deposition could have contributed to the changes observed in α6 integrin activation, this experiment was repeated on plates pre-coated with LN-1 (10 μg/ml). Plating on LN-1-coated substrates increased α6-integrin heterodimer levels in LEC (Fig. 5D), data indicating that SPARC-mediated LN-1 deposition contributes in part to α6-integrin heterodimer formation.

We also investigated whether intracellular focal adhesion-associated proteins in LEC were affected in the absence of SPARC. During

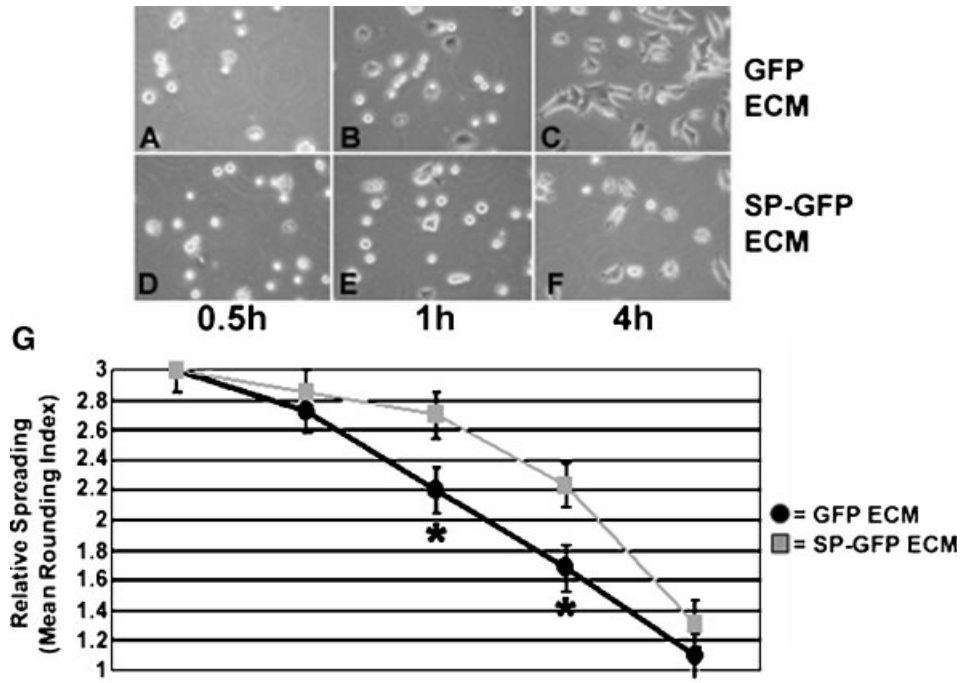

Fig. 3. SPARC-mediated ECM production contributes to differential LEC adhesion. SPARC-null LEC, transfected with GFP alone or with SPARC-GFP vectors, were incubated in serum-free DMEM for 72 h. Plates were washed to remove cells, re-seeded with untransfected SPARC-null LEC in 0.5% FBS/DMEM, and incubated for the times indicated. SPARC-null cells plated on ECM deposited by GFP-transfectants (**A**–**C**) or by SPARC-GFP-transfectants (**D**–**F**) were photographed at the indicated time-points. **G**: A Rounding Index was calculated from randomly-selected fields (**panels** A–F). Data represent means ± standard deviation of three experiments, and each point represents >500 cells. * $P < 0.02$.

adhesion, various scaffolding and intermediate signaling proteins co-localize at focal adhesions, providing mechanical connections to the cytoskeleton and transduction for intracellular signaling pathways. Adherent SPARC-GFP-expressing and GFP-expressing LEC were incubated in serum-free DMEM for 24 h, and were subsequently released by trypsin and re-plated under serum-free conditions for 2–4 h. Cells were analyzed for the expression of the focal adhesion-scaffolding protein talin during early stages of adhesion (Fig. 6A). Levels of talin in GFP-LEC were elevated relative to SPARC-GFP-expressing LEC. Previous work has shown that talin-mediated focal adhesion formation is upregulated upon the interaction of talin with the β1 integrin cytoplasmic tail [Cram and Schwarzbauer, 2004]. Co-immunoprecipitation of whole cell lysates was, therefore, performed with an anti-β1 integrin antibody. Probing for talin showed enhanced β1 integrin-talin interaction in cells lacking SPARC (GFP, Fig. 6B).

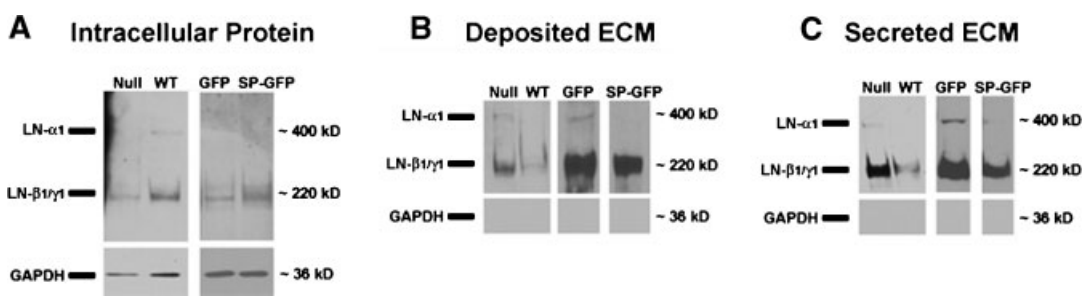

Fig. 4. Secretion/deposition of LN-1 by LEC. SPARC-null, WT, GFP-expressing (GFP), and SPARC-GFP-expressing (SP-GFP) LEC were plated at equal cell numbers and incubated with 0.1% BSA serum-free DMEM for 72 h. **A**: Cell lysates (intracellular protein) (20 μg per lane) from SPARC-null, WT, GFP-transfected, and SPARC-GFP-transfected cells; (**B**) Deposited ECM, and (**C**) concentrated conditioned medium (Secreted ECM) were analyzed by Western blot with anti-LN1 and anti-GAPDH antibodies. Molecular weights in kD are shown on the right of each panel.

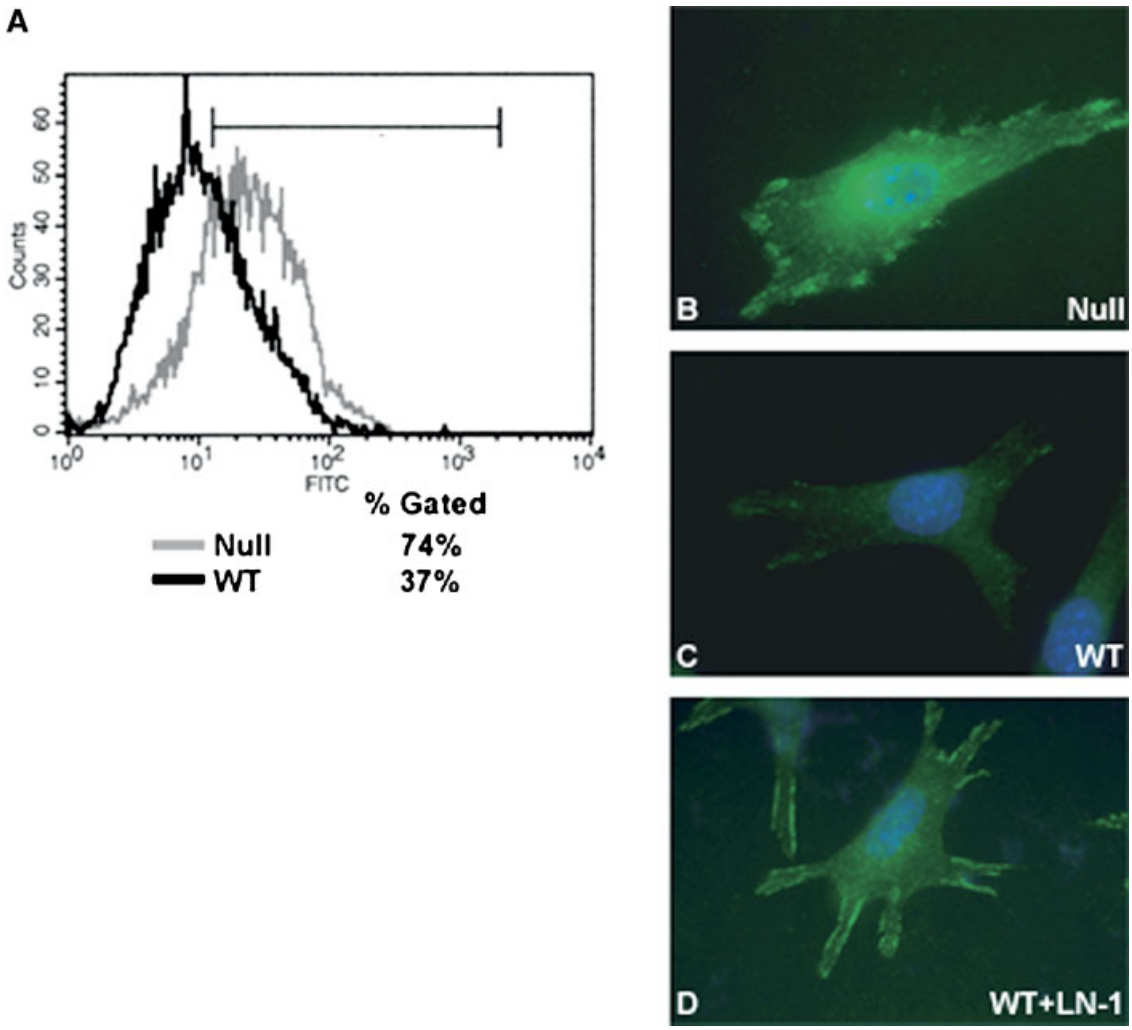

Fig. 5. α6-integrin heterodimers are increased in SPARC-null LEC. SPARC-null and WT cells were grown in 0.5% FBS/DMEM for 24 h, detached, stained with anti-α6 integrin IgG, and measured by flow cytometry. **A**: α6-integrin heterodimers in SPARC-null (gray line) versus WT LEC (black line). Values indicate positive α6 expression in each population. The gate was set by an isotype control for non-specific fluorescence. SPARC-null LEC (**B**), WT LEC (**C**), and WT LEC plated on a LN-1-coated dish (10 μg/ml) (**D**) were cultured for 24 h and immunostained for α6-integrin heterodimers. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

This finding correlates with our unpublished data showing increased focal adhesion formation in SPARC-null LEC.

The clustering of intracellular signaling proteins at focal adhesions allows for a large number of divergent signals to be initiated during adhesion. Probing for changes in the phosphorylation status of several of these proteins revealed a onefold increase in paxillin (Fig. 6C) in GFP-control cells. Comparison of suspension (0 h) and early adhesion (1 h) conditions revealed that the phosphorylation of paxillin appeared sensitive to the adhesion status and the presence of SPARC (Fig. 6C). Paxillin acts as an adaptor protein during adhesion; its activity is regulated by phosphorylation and association with focal adhesions [Schaller, 2001]. We conclude that increased levels of talin associated with β1-integrin and enhanced levels of Tyr-phosphorylated paxillin were apparent in SPARC-negative LEC (GFP) relative to SPARC-positive LEC (SP-GFP) during the early stages of adhesion.

DISCUSSION

Using cultured LEC expressing or lacking endogenous SPARC, we report that the absence of SPARC was associated with enhanced adhesion that in turn was correlated with increases

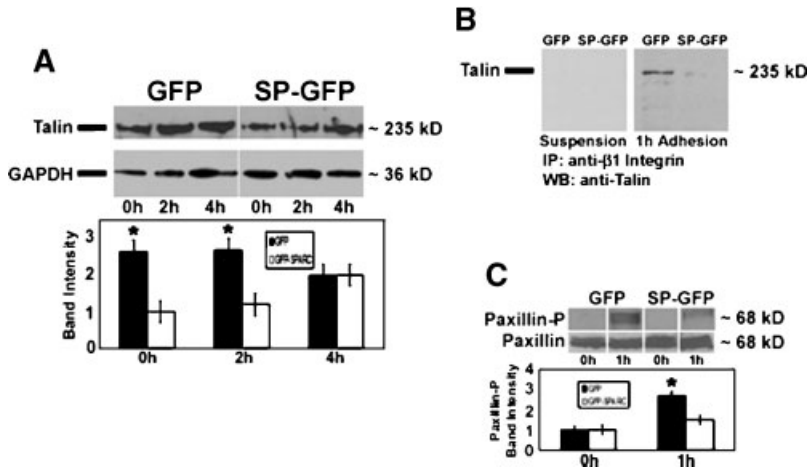

Fig. 6. Increased activity of focal adhesion-associated proteins in GFP- versus SPARC-GFP-expressing LEC. SPARC-null LEC expressing GFP or SPARC-GFP were plated in 10% FBS/DMEM for 24 h, washed, and incubated for an additional 24 h in serum-free DMEM. Cells were detached and re-plated in serum-free DMEM for the times indicated, prior to analysis. Western blotting was performed with antibodies against talin (**A**) and paxillin (**C**), and an antibody specific for phosphoTyr (C) (paxillin-P). Lysates were immunoprecipitated with anti-β1-integrin IgG and were probed for talin (**B**). Molecular weights in kD are indicated on the right of each panel. Data were normalized to an internal loading control (GAPDH and total paxillin) and represent means ± standard deviation of three experiments. $^*P < 0.02$.

in (1) focal adhesions, (2) secretion and deposition of the adhesive ECM proteins LN-1 and FN, (3) levels of α6-integrin heterodimers, and (4) talin and levels of Tyr-phosphorylated paxillin.

The lenses of SPARC-null mice exhibited alterations in capsular composition and an abnormal interface between the capsular ECM and the underlying LEC [Norose et al., 2000; Yan et al., 2002, 2005b]. As SPARC is expressed in LEC, but is not a structural component of the lens capsule [Yan et al., 2003], it has been postulated to modulate the morphology and function of the epithelial cells. Although previous work has shown SPARC to be a counter-adhesive protein on various non-lens cell types, the effect of SPARC on LEC-ECM interaction and LEC adhesion had been unknown and was the focus of this study.

ECM deposition and/or secretion affects the structural and functional characteristics of basement membranes. In the lens capsule, LN-1 has been shown to be a major structural component impacting the adhesion, migration, and survival of LEC [Oharazawa et al., 1999]. SPARC expression in LEC correlated with less LN-1 deposition and secretion in vitro (Fig. 4), consistent with an observation in vivo: increased levels of LN-1 were found in SPARC-null lens capsules relative to their WT counterparts, and there was a change in location of the final LN-1 heterotrimer [Yan et al., 2005b]. Yan et al. [2005b] also showed that SPARC was associated with LN-1 in the endoplasmic reticulum prior to secretion, an interaction that could be important in controlling the amount or quality of the LN-1 secreted into the ECM.

Integrins transduce signals from the ECM to the cytoplasmic face of the plasma membrane at focal adhesion sites [Clark and Brugge, 1995]. α6-integrin heterodimers in LEC focal contacts (Fig. 5B,C) were increased in the absence of SPARC. This change in α6-integrin heterodimers was more likely a downstream effect of the enhanced LN-1 deposition, since addition of LN-1 to the plates enhanced the levels of α6-integrin heterodimers (Fig. 5D). α6-integrin has been shown to play a role in normal lens function [Walker et al., 2002] and has a specific function in the differentiation of LEC into fiber cells. Therefore, activation of this integrin is likely to contribute to the balance between adhesion/migration and differentiation of LEC in the SPARC-null lens.

Talin, a major focal adhesion scaffolding protein, is a vital component for focal adhesion formation as well as subsequent downstream signaling [Nayal et al., 2004]. Interaction between talin and the β1-integrin cytoplasmic tail has been linked to augmented adhesion. Our observations here support this claim in LEC. SPARC-null LEC had increased talin levels under suspension conditions (unattached cells) and during early stages of attachment (2 h) (Fig. 6A). When activated by phosphorylation

during adhesion, paxillin is associated with focal adhesions and cytoskeletal (F-actin) components [Brown and Turner, 2004]. Phosphorylation of paxillin has been shown to mediate both adhesion and, alternatively, de-adhesion [Parsons and Parsons, 1997; Schaller, 2001]. Recent studies have raised the possibility that Tyr-phosphorylation of paxillin leads to its dissociation from focal adhesions, ubiquitinization, and subsequent degradation [Didier et al., 2003; Huang et al., 2004]. It follows that a counter-adhesive protein such as SPARC would downregulate phosphorylation of paxillin during early stages of adhesion (1 h), as demonstrated in Figure 6C. In contrast to our findings, Young et al. [1998] reported that purified SPARC added to the culture medium, enhanced paxillin phosphorylation in bovine aortic endothelial cells. This discrepancy could be attributed to the different cell types and the activity/location of exogenous versus endogenous SPARC protein.

In summary, we have demonstrated that SPARC exerts a counter-adhesive function on cultured LEC and have identified multiple factors that contribute to this important characteristic. An understanding of how SPARC functions in LEC will elucidate how a matricellular protein contributes to the maintenance of lens transparency. The consequences of changes in epithelial-ECM interaction significantly influence lens epithelial homeostasis.

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