Alterations in the lens capsule contribute to cataractogenesis in SPARC-null mice

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Summary

The lens capsule, which is also called the lens basement membrane, is a specialized extracellular matrix produced anteriorly by the lens epithelium and posteriorly by newly differentiated fiber cells. SPARC (secreted protein, acidic and rich in cysteine) is a matricellular glycoprotein that regulates cell-cell and cell-matrix interactions, cellular proliferation and differentiation, and the expression of genes encoding extracellular matrix components. SPARC-null mice exhibit lens opacity 1 month after birth and mature cataract and capsular rupture at 5-7 months. In this study, we report disruption of the structural integrity of the lens capsule in mice lacking SPARC. The major structural protein of basement membrane, collagen type IV, in the lens capsule was substantially altered in the absence of SPARC. The lens cells immediately beneath the capsule showed aberrant morphology, with numerous protrusions into the lens basement membrane. SPARC-null lenses at 1 month of age exhibited an increased penetration of dye or radioactive tracer through the capsule, as well as a higher content of water than their wild-type counterparts. Moreover, SPARC-null fibers exhibited swelling as early as 1 month of age; by 3 months, all the fiber cells appeared swollen to a marked degree. By contrast, the absence of SPARC had no apparent morphological effect on the early stages of lens formation, cell proliferation or fiber cell differentiation. Degradation of crystallins and MIP 26, or changes in the levels of these proteins, were not detected. These results underscore the importance of the capsular extracellular matrix in the maintenance of lens transparency and indicate that SPARC participates in the synthesis, assembly and/or stabilization of the lens basement membrane.

Key words: SPARC, Lens capsule, Basement membrane, Extracellular matrix, Epithelial cells, Fiber cells, Collagen IV, Cataract, Permeability

Introduction

Targeted disruption of the *SPARC* (secreted protein, acidic and rich in cysteine) gene has been performed in mice from three different genetic backgrounds (Gilmour et al., 1998; Norose et al., 1998). The mice are viable and fertile and appear grossly similar to their wild-type (wt, $^{+/+}$) counterparts. Unexpectedly, a predominant and consistent phenotype of all SPARC-null ($^{-/-}$) mice is the presence of cataracts. The mechanism of cataractogenesis in SPARC-null mice is not known. In this report, we provide a partial explanation for this pathology.

SPARC, which is also termed osteonectin or BM-40, belongs to the matricellular class of secreted glycoproteins (thrombospondins 1 and 2, osteopontin, tenascins C and X) that function as modulators of cell-cell and cell-matrix interactions. SPARC mediates these interactions by (1) binding to extracellular matrix (ECM) proteins, (2) regulating ECM and growth factor production/efficacy and/or 3) modulating matrix metalloproteinase expression (Lane and Sage, 1994; Tremble et al., 1993; Yan and Sage, 1999; Sage, 1997). Both counteradhesive and antiproliferative properties in vitro have been attributed to SPARC, which induces rounding of cultured cells and disassembly of focal adhesions (Lane and Sage, 1990; Murphy-Ullrich et al., 1995). SPARC participates in morphogenesis, tissue repair and differentiation by virtue of its regulation of cell cycle, cell shape change, migration, adhesion and ECM production (Lane and Sage, 1994; Yan and Sage, 1999). Therefore, SPARC does not appear to serve a structural role in the ECM but is a functional modulator of various activities attributed to protein/proteoglycan networks (Sage and Bornstein, 1991; Bornstein, 1995). Despite its expression as a consequence of development or injury-related remodeling (Brekken and Sage, 2001), the production of SPARC in normal adult tissues is rather limited. Interestingly, SPARC is expressed in lenticular epithelium in both developing and adult mammals (Yan et al., 1998; Yan et al., 2000). The phenotype of early cataract formation in SPARC-null mice indicates that SPARC participates significantly in the function and homeostasis of the normal lens.

The lens is a cellular structure without blood vessels, lymphatics and nerves. It is enclosed by an avascular thick capsule and is nourished by diffusion from the aqueous humor through the lens capsule and epithelium. The capsule, also called the lens basement membrane (BM), is a substantial, acellular and structurally complex ECM, consisting of an orderly meshwork of various glycoproteins and proteoglycans (for example, collagen type IV, laminin, perlecan, nidogen, and fibronectin) (Timpl and Dziadek, 1986; Bosman et al., 1989; Cammarata et al., 1986). BM produced by different cells has been correlated with functions such as proliferation, differentiation, adhesion and permeability (Yurchenco and

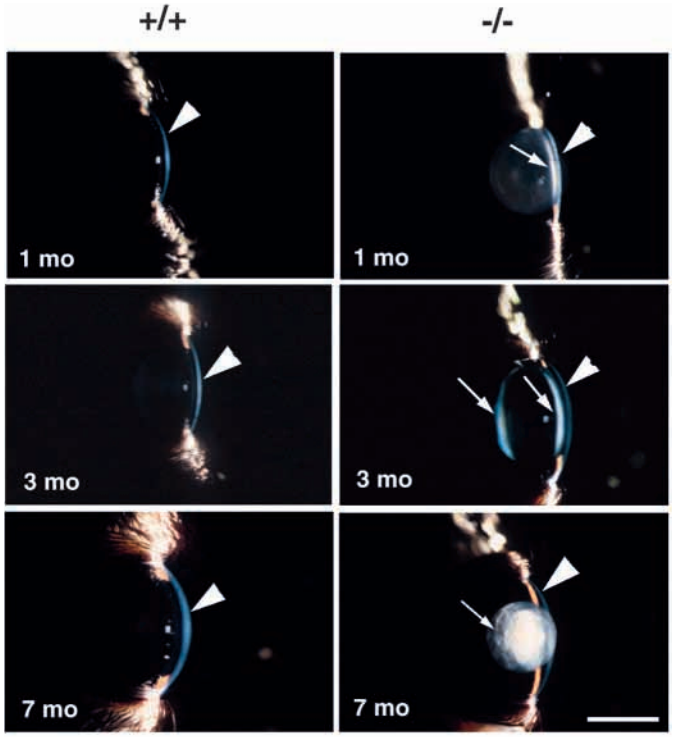

Fig. 1. Absence of SPARC results in cortical cataract. Slit-lamp photographs of wt mice ($^{+/+}$) at 1, 3 and 7 months of age and SPARC-null mice ($^{-/-}$) at 1, 3 and 7 months are shown. The SPARC-null lens at 1 month shows anterior subcapsular cortical opacity (arrow). SPARC-null lens at 3 months displayed anterior and posterior subcapsular opacity (arrows), whereas the nucleus is transparent. The SPARC-null lens at 7 months has a mature cataract (arrow). Arrowheads indicate the cornea, and arrows indicate lens opacity. mo, month. Bar, 240 μm.

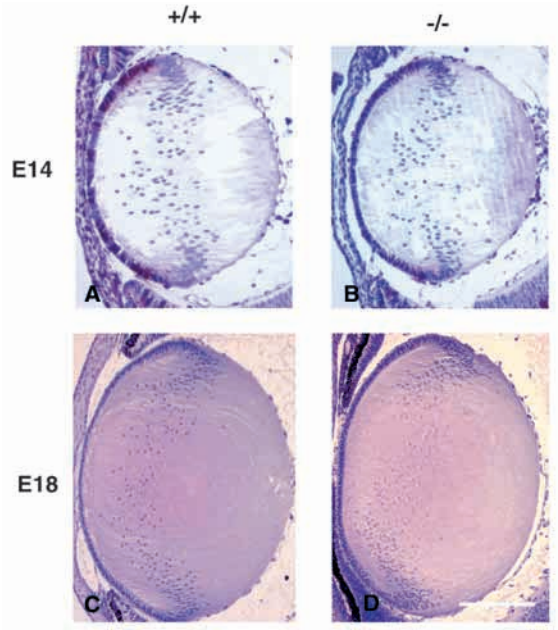

Fig. 2. Histology of SPARC-null lens at E14 and E18. Sections of wt and SPARC-null lenses were stained with hematoxylin and eosin and were examined by light microscopy. The sizes of the SPARC$^{+/+}$ and SPARC-null ($^{-/-}$) lenses at E14 and E18 are highly similar. The primary lens fibers have elongated to contact the anterior epithelium, and the lens vesicle lumen has disappeared at E14. The $^{-/-}$ lens fiber elongation is complete, and there are no vacuoles in the $^{-/-}$ lenses. Bar (A,B), 270 μm; bar (C,D), 400 μm.

Schittny, 1990). Thus, the composition of the ECM can be expected to be of particular importance for the maintenance of normal morphological and functional properties of the lens. Other important functions of the lens capsule that are dependent upon the organization of its constituent proteins and proteoglycans are filtration and permeability (Fisher, 1977; Lee et al., 1997; Winkler et al., 2001). The capsular ECM of the lens is not a static structure; rather, it is continually produced and remodeled anteriorly by the lens epithelial cells and posteriorly by newly differentiated fiber cells (Johnson and Beebe, 1984). SPARC regulates the production of certain ECM proteins, in addition to its interaction with collagens (Sasaki et al., 1998; Sage et al., 1989; Mayer et al., 1991; Maurer et al., 1995) and growth factors (Raines et al., 1992; Kupprion et al., 1998). In this study we have asked whether SPARC plays a significant role in the organization and deposition of ECM proteins in the lens capsule and in its structural integrity.

This report demonstrates alterations in the structure of the lens capsule, increased dye or radioactive tracer penetration through the capsule and swelling of the lens fiber cells in lenses from mice with a targeted disruption of the SPARC gene. The absence of SPARC disturbed the normal relationship between the capsular ECM and the underlying cells. We propose that damaged capsular integrity contributes significantly to cataractogenesis in SPARC-null mice and that cell-matrix interactions that are sensitive to the presence (or diminution) of SPARC are a major component of cataractogenesis. The SPARC-null mouse appears to be an opportune model for understanding the role of SPARC in the modulation of ECM organization and function.

Materials and Methods

Mice

Targeted disruption of the *SPARC* gene was performed in embryonic stem cells from 129SvEv mice (Norose et al., 1998), and these cells were injected into C57BL/6J blastocysts to generate chimeras. F1 SPARC heterozygous mice were produced by the mating of male chimeras with C57BL/6J females (Norose et al., 1998). Crosses between heterozygotes produced a Mendelian distribution of homozygous ($^{-/-}$), heterozygous ($^{+/-}$) and wt animals ($^{+/+}$). The homozygous mutants expressed neither SPARC mRNA nor protein. A polymerase chain reaction (PCR) method, as described previously (Bassuk et al., 1999), was used to genotype the transgenic mice. The presence of the vaginal plug was counted as day 0 of gestation (E0). All the mice used in this study were maintained under pathogen-free conditions. The treatment and use of mice followed the guidelines for the Care and Use of Laboratory Animals by the National Institutes of Health and the Association for Research in Vision and Ophthalmology Statement for the use of Animals in Ophthalmology and Vision Research.

Slit lamp examination

Lenses of both wt and mutant mice were examined and photographed by slit-lamp photomicroscopy (Nikon FS-2). The pupils of unanesthetized mice were dilated with 0.1% Mydriacyl tropicanide

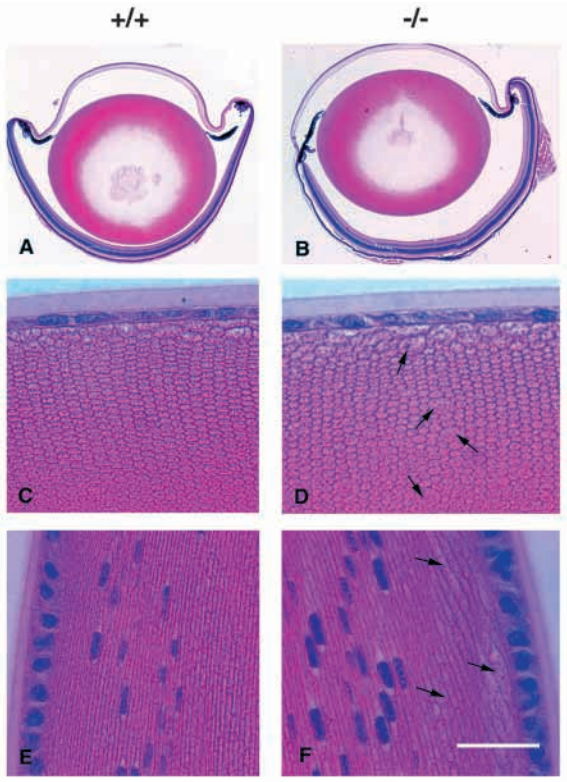

Fig. 3. Histology of SPARC-null lens at 1 month of age. Sections of wt and SPARC-null lenses were stained with hematoxylin and eosin. Comparable regions of wt (A,C,E) and SPARC-null lenses (B,D,F) are shown. (C,D) center anterior region of cortex; (E,F) equatorial (bow) region of lens. Note that $^{+/+}$ and SPARC-null ($^{-/-}$) lenses are grossly indistinguishable, with the lens nucleus centrally located (A,B). A few of the fiber cells in the SPARC-null ($^{-/-}$) lens show altered morphology (arrows in D and F). Bar (A,B), 540 μm; bar (C-F), 36 μm.

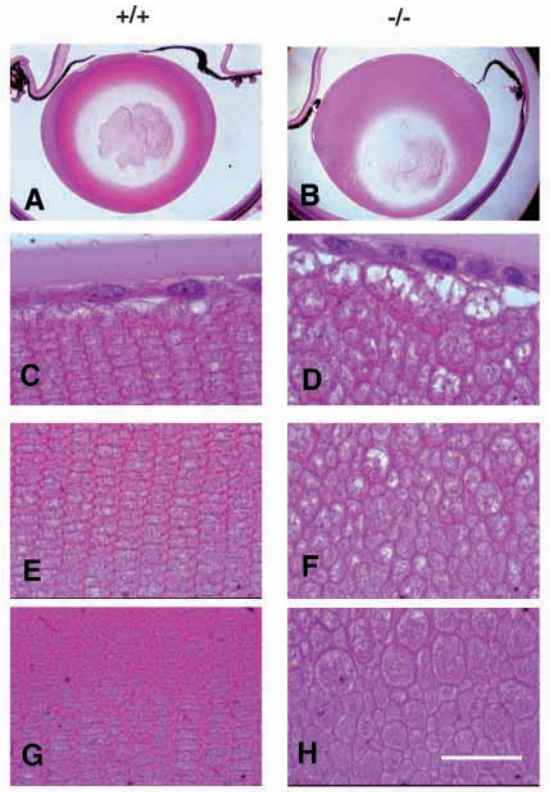

Fig. 4. Histology of SPARC-null lens at 3 months of age. Sections of wt and SPARC-null lenses were stained with hematoxylin and eosin. Loss of normal shape of secondary fiber cells in SPARC-null lenses is shown. Comparable center cortical anterior regions from $^{+/+}$ (A,C,E,G) and SPARC-null ($^{-/-}$) lenses (B,D,F,H) are shown. Note that in the SPARC-null lens, most of the secondary fiber cells have become rounded and swollen, and the nucleus has been displaced toward the posterior capsule (B). Bar (A,B), 540 μm; bar (C-H), 18 μm.

ophthalmic solution and 10% phenylephrine hydrochloride ophthalmic solution (1:1 by volume). Slit views were taken at a 30° angle to the optic axis with a Nikon electronic flash power supply at a maximum setting.

BrdU delivery, histology, immunohistochemistry and EM

Pregnant mice were given 100 μg BrdU (5-bromo-2′-deoxyuridine; Sigma, St Louis, MO) suspended in phosphate-buffered saline (PBS) by intraperitoneal injection. One hour after the injection, embryonic eyes were collected. Postnatal mice younger than 2 months of age and weighing less than 20 g were injected with 500 μg BrdU. The eyeballs were collected 2 hours after injection. For mice older than 2 months and weighing more than 20 g, BrdU was loaded into an osmotic minipump (Alza, Palo Alto, CA) implanted under the skin behind the interscapular space. BrdU was delivered at a rate of 2 μg/g body weight per hour for 1 week to ensure an identical amount infused per unit of body weight (Li et al., 1997). The collected eyeballs were fixed immediately in 10% neutral buffered formalin (0.1 M sodium phosphate, pH 7.4), dehydrated through a series of ethanol concentrations and embedded in paraffin. Serial 5 μm thick paraffin sections were cut medially and through the optic nerve head. Immunostaining with anti-BrdU antibody was performed as described previously (Li et al., 1997).

BrdU incorporation into lens epithelium was assessed by microscopy. Nine sections of each lens per animal were analyzed, and an average value was determined. Three to six animals were studied for each time point. All counts were performed without knowing the identity of the animals. Wild-type and transgenic mice were compared within the same age group. Significant differences were determined by Student's paired *t*-test for comparison of two sample means.

Lenses from embryos and postnatal mice were prepared by fixation with methyl Carnoy's solution (60% methanol, 30% chloroform and 10% glacial acidic acid) for 4 hours. The eyeballs were dehydrated in a solution of ethanol and were embedded in paraffin for staining with hematoxylin and eosin and for immunofluorescence (anti-mouse collagen IV(α1/α2) IgG, Collaborative Biomedical Research, Bedford, MA). For reaction with the anti-MIP26 antibody, the eyeballs were fixed with 4% paraformaldehyde for 20 minutes, washed with PBS and soaked for 4 hours in 30% sucrose in PBS. Frozen sections were processed and exposed to anti-MIP (major intrinsic protein) IgG, followed by a secondary antibody conjugated with fluorescein isothiocyanate. For electron microscopy (EM), lenses were fixed in 2.5% glutaradehyde in 0.1M sodium cacodylate buffer and were processed and photographed as described (Wight et al., 1997; Norose et al., 2000).

Detection of lens proteins and mRNA

Before sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), each lens nucleus was separated from the cortex. The cortex was extracted with 0.1 M NaCl. The supernatant produced represents the total cortical water-soluble proteins. The insoluble pellet was extracted further with 8 M urea; this supernatant represents

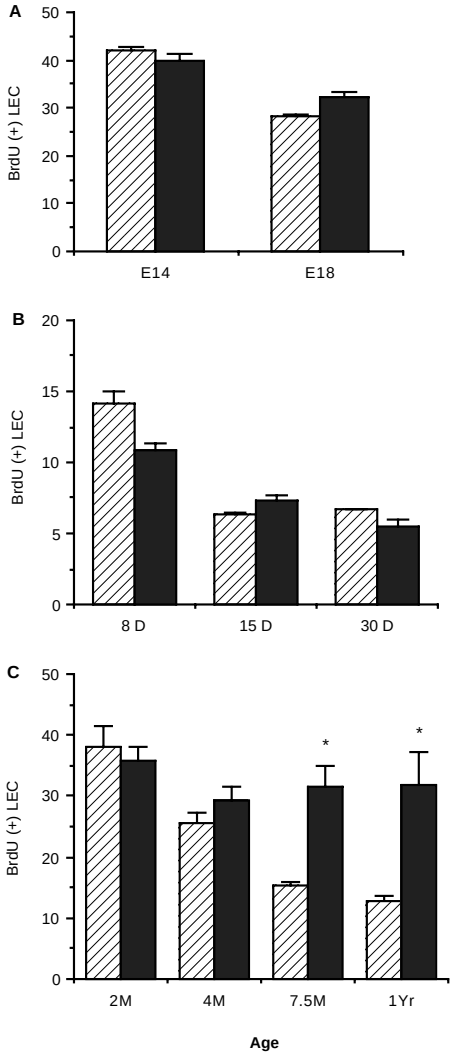

Fig. 5. No significant change in cell proliferation in the lens epithelium between wt and SPARC-null lenses, prior to mature cataract formation, was observed. (A) Pregnant mice were injected with 12.5 mg BrdU for 1 hour before sacrifice of the embryos. (B) Postnatal mice were injected with 2.5 mg BrdU for 2 hours before sacrifice. (C) Mice weighing in excess of 20 g received BrdU by minipump delivery (2 μg/g body weight/hour for 1 week). Hatched bars, wt lenses; filled bars, SPARC-null lenses. BrdU-labeled cells (+) were counted in nine sections per animal. Three to six mice were analyzed for each time point. DNA synthesis in SPARC-null mice increased when the cataract was mature and when inflammation was associated with rupture of the lens capsule (7.5 months and 1 year-old). D, day; M, month; Yr, year; LEC, lens epithelial cells. *, $P<0.01$.

the urea-soluble proteins in the cortex, and the final insoluble pellet was termed the urea-insoluble pellet. The same procedure was performed on the lens nucleus, with the resulting three fractions described above. All samples were dissolved in sample buffer (60 mM Tris [pH6.8], 2%SDS, 10% glycerol, and 0.001% bromphenol blue) containing 10 mM dithiothreitol (DTT) and were boiled for 5 minutes. 4-20% precast polyacrylamide gradient gels (Novex) were used for SDS-PAGE, and proteins were stained with Coomassie Brilliant blue.

For immunoblotting, lenses were homogenized in 0.1M NaCl, 0.1 M Na_2HPO_4 (pH 7.4). Soluble and insoluble fractions were collected

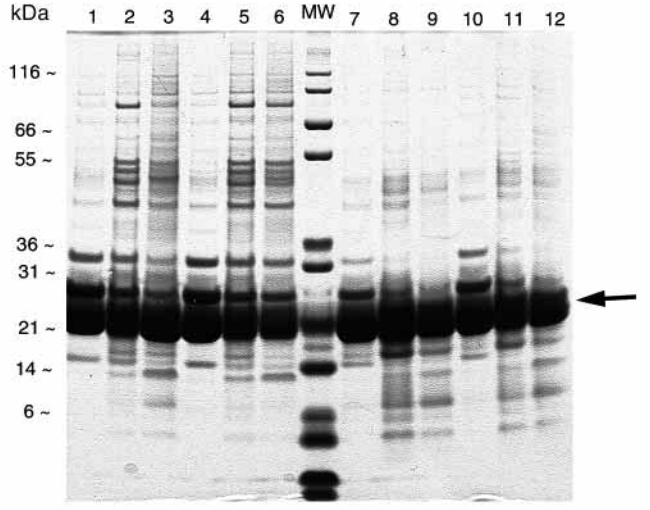

Fig. 6. Characterization of lens fiber proteins in 3-month-old wt and SPARC-null lenses. Lens fiber cells from $^{+/+}$ and SPARC-null ($^{-/-}$) animals were separated into cortical and nuclear fractions (see Materials and Methods). Proteins were extracted into water-soluble, urea-soluble and pellet (urea-insoluble) fractions and were resolved by SDS-PAGE under reducing conditions. Lanes 1, $^{+/+}$ water-soluble cortex; 2, $^{+/+}$ urea-soluble cortex; 3, $^{+/+}$ pellet cortex; 4, $^{-/-}$ water-soluble cortex; 5, $^{-/-}$ urea-soluble cortex; 6, $^{-/-}$ pellet cortex; molecular weight markers (kDa); 7, $^{+/+}$ water-soluble nucleus; 8, $^{+/+}$ urea-soluble nucleus; 9, $^{+/+}$ pellet nucleus; 10, $^{-/-}$ water-soluble nucleus; 11, $^{-/-}$ urea-soluble nucleus; 12, $^{-/-}$ pellet nucleus. The different fractions between $^{+/+}$ and $^{-/-}$ lenses exhibit similar patterns, with no alteration of the major crystallins (arrow).

and dissolved in sample buffer with 10 mM DTT prior to SDS-PAGE and subsequent transfer (Yan et al., 2000).

Total RNA was isolated from lens fiber cells of wt and SPARC-null mice with an RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Lens fiber cells were homogenized in TRI reagent (Molecular Research Center, Inc., Cincinnati, OH) and were washed initially with chloroform and isopropanol to remove fiber proteins. The quality and yield of recovered RNA were evaluated by absorption at 260 and 280 nm. Total RNA was reverse-transcribed into cDNA by the use of an Omniscript RT kit (Qiagen). MIP cDNA was amplified using the primer pair: GCCTGGCCTTGGCTACAT-TGGT/TGGCCTTGGCTACATTGGT.

Lens capsular permeability assay and measurement of water content

Whole lenses with intact capsules were removed from freshly enucleated eyes under a dissecting microscopy. Lenses from wt and SPARC-null mice were incubated immediately in 0.4% trypan blue dye (Sigma), 1 μCi/ml [methyl-^{3}H]-thymidine (1 mCi/ml; New England Nuclear, Boston, MA) or a 1: 2000 dilution of 1 mCi/g ^3H_2O (1 mCi/g; New England Nuclear, Boston, MA) for 20 minutes at room temperature. The lenses were subsequently rinsed briefly in PBS. The lens capsules were removed immediately. The decapsulized lenses and isolated capsules were homogenized separately (1 lens capsule/100 μl PBS; 1 decapsulized lens/100 μl PBS in a 1.5 ml tube). The [^{3}H]-thymidine or ^3H_2O that penetrated into the lens capsules or lens masses was quantified by liquid scintillation counting in 3 ml Ecolume (ICN, Irvine, CA). For quantification of trypan blue dye, supernatants were collected after centrifugation of the lens capsules or lens masses at 13,000 ***g***, and the absorbance at 497 nm was determined for samples.

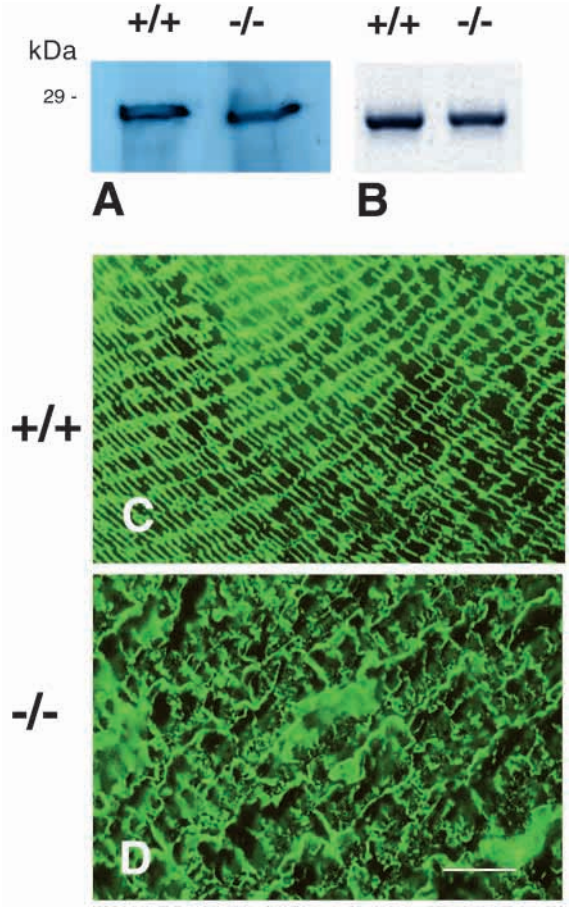

Fig. 7. Expression of MIP in SPARC-null lens. **(**A) An immunoblot of SPARC$^{+/+}$ and SPARC-null ($^{-/-}$) lens fiber lysates (from 3 month-old lenses) with antibody against MIP 26. (B) RT-PCR of lens fiber cells (3 month-old) with MIP 26 primers. MIP 26 protein and mRNA are present in both fiber cell samples, with minimal differences. (C,D) Cross-sections through the lens cortex showing 2-month-old lens fiber cells stained with antibody against MIP 26 in $^{+/+}$ (C) and $^{-/-}$ lenses (D). The closely packed organization of lens fibers is disrupted in 2-month-old lenses. Bar (C,D), 18 μm.

Intact lenses (1 month) were weighed immediately after dissection from the eyeballs (wet weight). Each lens was dried in an oven at 90°C for 16 hours and was weighed again (dry weight).

Results

SPARC-null mice develop a cortical cataract

We examined lenses of age-matched wt and SPARC-null mice from 3 weeks to 1 year of age by slit-lamp microscopy. The SPARC-null mice showed 100% penetrance of the cataract, whereas no significant opacification was observed in the lenses of wt animals up to 1 year of age. Lenticular opacity could be detected in some of the SPARC-null mice in the anterior and/or posterior subcapsular cortex as early as 4 weeks after birth [Fig. 1, 1 month (mo), arrow indicates anterior opacity]. The degree and extent of the cortical opacity were progressive and varied among individual mice of the same age, as well as in both lenses of the same mouse (data not shown). By 3 months of age, the SPARC-null lenses often showed both anterior and posterior subcapsular cortical opacity, whereas the nucleus was clear [Fig. 1, 3 months (mo), arrows]. By 5-7 months of age, opacity was present in the nuclear region, with complete loss of lens transparency [i.e., full mature cataract, Fig. 1, 7 months (mo), arrow].

Cataract lens morphology in SPARC-null mice

To determine the onset of cataract formation in SPARC-null mice, we examined lenses from SPARC$^{+/+}$ and SPARC-null animals before and after the appearance of cataracts. In E14 and E18 embryos, wt and SPARC-null lenses were indistinguishable in size and in general morphology (Fig. 2). The initial induction of the lens vesicle on E11.5 was not affected (data not shown). Elongation of the lens fiber cells appeared to proceed normally, as the cavity of the lens vesicle was filled with postmitotic, differentiated fiber cells (Fig. 2). The nuclei of the lens fiber cells were located in the anterior part of the cytoplasm. Microphthalmia was not detected during the development of SPARC-null mice. At 1 month (the beginning of cortical opacity), the SPARC-null lenses exhibited the same size and symmetrical arrangement of lens fibers, with the lens nucleus centrally located (Fig. 3A,B). However, under higher magnification, a few of the cortical fiber cells of the SPARC-null lenses appeared enlarged/swollen and lost their hexagonal shape (Fig. 3D, arrows). Tiny vacuoles appeared in fibers at the bow region; these fiber cells were loosely packed and exhibited an uneven distribution of cytoplasm (Fig. 3F, arrows). By 3 months of age, the size of wt and SPARC-null lenses remained similar (Fig. 4A,B). However, the SPARC-null lens nucleus was displaced posteriorly (Fig. 4B). All the secondary fiber cells in SPARC-null cortex were swollen, altered in size and shape and disorganized (Fig. 4D,F,H). By 4 months, SPARC-null lens fiber cell swelling was advanced, fiber cell plasma membranes were ruptured and vacuolated fibers appeared at the bow region (Bassuk et al., 1999). At 5 months, the SPARC-null posterior lens capsule had ruptured, and the nucleus was dislocated in the posterior cavity, with a severely disorganized cortex (data not shown) (Norose et al., 1998).

Lens epithelial cell proliferation is not a causative factor in cataract formation

Whether or not the proliferation of lens epithelial cells is abnormal and a causative factor in the formation of cataract in SPARC-null lenses is not known. Lens development and growth depend on normal lens epithelial cell proliferation and terminal differentiation into lens fibers (Wride, 1996). Because SPARC is a potent inhibitor of the cell cycle in vitro, and overproliferation of lens epithelial cells is associated with posterior subcapsular cataract, lens cell proliferation was measured in SPARC$^{+/+}$ and SPARC-null lenses. Indirect immunocytochemical staining of BrdU-incorporated S-phase cells was conducted in lenses from E14 to 1 year old SPARC$^{+/+}$ and SPARC-null mice. Detection of BrdU incorporated into DNA was used as an indication of replicating cells. From E14-E18, the primary fiber cells formed normally, and cell proliferation was restricted to the anterior lens epithelium, with equivalent numbers of BrdU-positive cells in SPARC$^{+/+}$ and SPARC-null animals (Fig. 5A). Inappropriate S-phase entry in lens fiber cells was not observed in SPARC-null embryonic

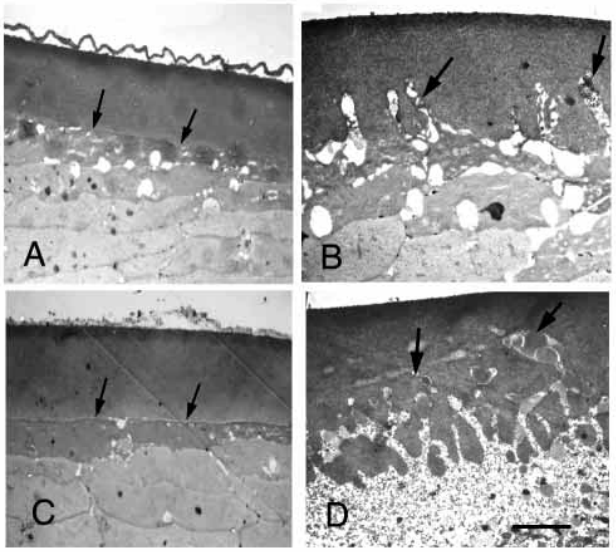

Fig. 8. Transmission EM of the lens capsule. (A) A 1-month-old SPARC$^{+/+}$ lens capsule posterior to the equator is shown. (B) 1-month-old SPARC-null ($^{-/-}$) posterior lens capsule from the same region as A, in which the cell surface of newly formed $^{-/-}$ lens fibers bordering the capsule protrudes into the lens capsule (arrows). (C) 3-month-old $^{+/+}$ lens, same area as shown in A. (D) 3-month-old $^{-/-}$ lens, same region as C. Note the progressive infiltration of cell processes into the capsule (D, arrows). Bar, 5 μm.

mice (data not shown). Posnatally, there was no significant difference in BrdU labeling between wt and SPARC-null lens cells (Fig. 5B,C); moreover, the fiber cells showed no incorporation of BrdU up to 6-7 months of age. In 7.5 month-old SPARC$^{+/+}$ lenses, BrdU labeling was restricted mainly to the germinative zone of the epithelium, with a few in the transition zone, and there were reduced numbers of positive cells in comparison with younger SPARC$^{+/+}$ lenses. However, the number of BrdU-labeled cells increased significantly in 7.5 month-old SPARC-null lenses (P<0.01, Fig. 5C), largely because of fibroblast-like cell masses appearing beneath the posterior capsule (normally, nucleated cells are not found under the posterior capsule). The posterior capsule was thin and ruptured at this age. Ocular inflammation was indicated by the presence of neutrophils, lymphocytes and macrophages in the vitreous cavity (data not shown) (Norose et al., 1998). In 7 month to 1 year-old SPARC$^{+/+}$ mice (Fig. 5C), the extent of lens cell proliferation decreased. In contrast, SPARC-null lenses showed increased numbers of BrdU-labeled cells, concomitant with ocular inflammation. In summary, these results indicate that SPARC does not exert a significant effect on the cell cycle in the lens. Therefore, abnormal proliferation of lens epithelial cells does not contribute causally to cataractogenesis in animals lacking SPARC.

Disorganization of lens fibers in the absence of SPARC

Disorganization and swelling of lens fiber cells were the primary morphological alterations observed in the cortex of SPARC-null lenses at 1-3 months after birth. Since severe changes in fiber cells were apparent by 3 months of age (Fig. 4), we isolated these cells and analyzed the fiber proteins in wt and SPARC-null lenses. Fiber cells derived from both genotypes showed almost identical protein patterns by SDS-PAGE (Fig. 6). The water-soluble crystallin proteins (α, β and γ crystallins comprise approximately 90% of lens soluble proteins) showed no differences in levels or in degradation by immunoblot analysis of SPARC-null versus SPARC$^{+/+}$ lenses (data not shown). The MIP-26 (aquaporin 0) is a membrane-bound protein, abundantly and specifically expressed in lens fiber cells, that regulates the transport of water (Benedetti et al.,1974; Shiels and Bassnett, 1996). At either the mRNA or protein level, there were minimal differences between SPARC$^{+/+}$ and SPARC-null fiber cells with respect to expression of MIP26 (Fig. 7A,B). However, immunostaining for MIP26, which outlines the fiber cell membrane, revealed a striking disorganization of cortical fiber cells in SPARC-null relative to SPARC$^{+/+}$ lenses, which could result from swelling (Fig. 7C,D).

Since the secondary lens fiber cells were swollen by 2-3 months of age, and there was apparently no degradation or alteration of their major proteins, we began to suspect that the filtration barrier of the capsule and underlying cells for maintaining normal homeostasis might be compromised in the SPARC-null lens. Such a functional alteration could lead to an imbalance of water and ions in the lens and might result in swollen fibers as observed in Figs 3 and 4.

Compromised BM in SPARC-null lens

By light microscopy, the lens capsule showed minimal abnormalities within 3 months (Figs 3 and 4). Moreover, EM showed a smooth interface between the lens capsule and the underlying cells in SPARC$^{+/+}$ lenses at 1 and 3 months of age (Fig. 8A,C, arrows). However, by 1 month of age, SPARC-null lens fibers immediately posterior to the equator exhibited a few finger-like protrusions into the lens capsule (Fig. 8B, arrows). These protrusions appeared progressive, in that numerous large extensions penetrated deeply into the lens capsule at 3 months of age (Fig. 8D, arrows). These observations are in agreement with a previous EM study (Norose et al., 2000). The extensions of the cell membrane into the anterior capsule were not as obvious as those into the posterior capsule in the lenses of 1 month-old SPARC-null mice (data not shown).

Collagen type IV is a major structural component of the lens capsule (Fitch et al., 1983; Cammarata et al., 1986). Immunohistochemical staining of collagen IV revealed a similar distribution of this ubiquitous BM protein in SPARC$^{+/+}$ and SPARC-null anterior lens capsules of 1 month-old mice (Fig. 9A,C), However, SPARC-null lens capsules posterior to the bow region were abnormal (Fig. 9D, arrows). At 3 months, SPARC-null anterior lens capsules exhibited a more mottled appearance, and small, fine protrusions could be observed by light microscopy (Fig. 9G, arrows). The posterior lens capsule was characterized by numerous clefts, which penetrated deeply into the lens capsule and might be predicted to disrupt the permeability of the capsule (Fig. 9H, arrows).

Increased penetration of dye and radioactive tracer in SPARC-null lens

Trypan blue dye (906 Da) and [^{3}H]-thymidine (242 Da) were chosen as tracers to evaluate the penetration of small molecules

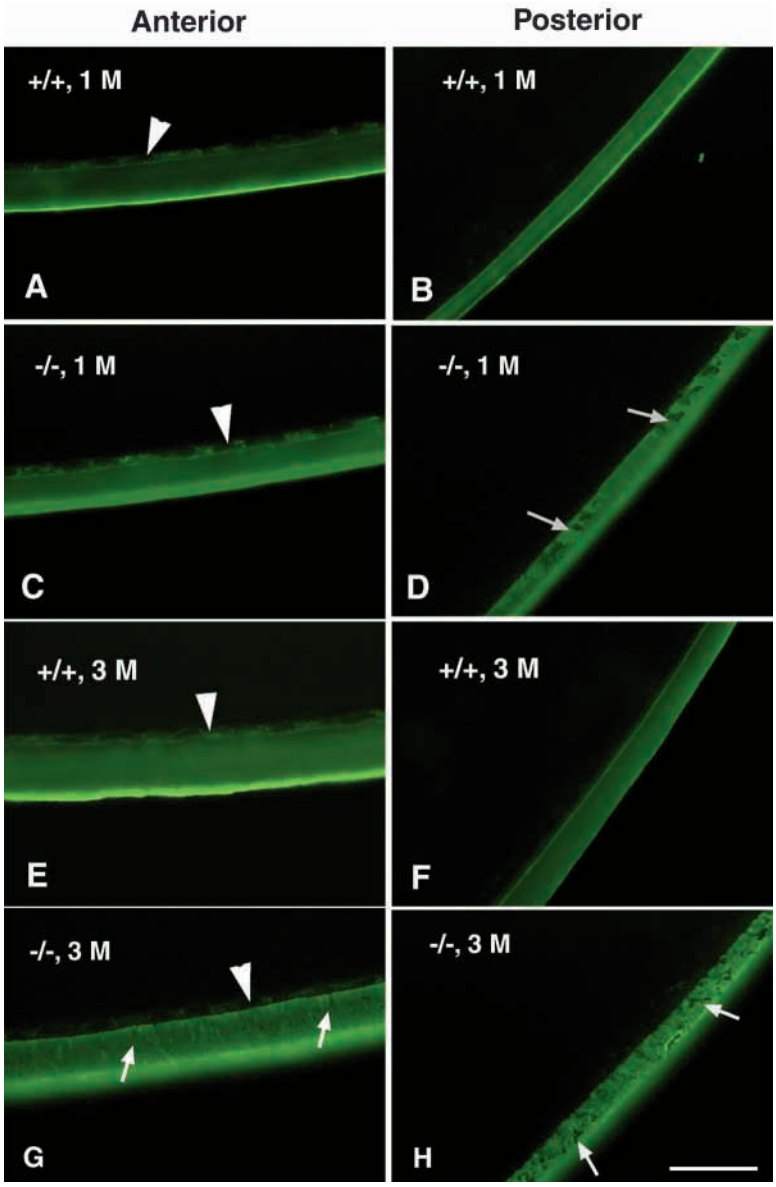

Fig. 9. Distribution of collagen IV in the lens capsule. Lenses were incubated with collagen IV IgG followed by a secondary antibody conjugated with fluorescein isothiocyanate. (A,C,E,G) anterior lens capsule; (B,D,F,H) posterior lens capsule. Arrows indicate the protrusions of lens cell processes into the SPARC-null ($^{-/-}$) capsules. Arrowheads indicate lens epithelial cells (stained lightly by anti-collagen IV IgG) underneath the capsules. Bar, 20 μm.

through the lens capsules of wt and SPARC-null lenses. In lenses from mice of 1-3 month of age, the possibility of incorporation of [^{3}H]-thymidine into lens epithelial cell DNA is highly unlikely during the 20 minute assay [there was no labeling of lens epithelial cells of 1-month-old mice after 1 half hour BrdU delivery; (Q.Y., unpublished)]. In Fig. 10 the difference in dye and [^{3}H]-thymidine penetration between wt and SPARC-null lenses is obvious. The dye content measured in SPARC-null decapsulized lenses (1-month-old) was 1.38 times the value of the SPARC$^{+/+}$ lenses. The [^{3}H]-thymidine CPM in SPARC-null decapsulized lenses were 1.3 times (1-month-old) and 3.4 times (3 month-old) the values of the corresponding +/+ lenses. ^3H_2O was also evaluated for its penetration into the lenses. After 20 minutes, the CPM in capsules of wt and SPARC-null mice (1 and 3 months of age) were minimal, with no difference (data not shown). However, there were significant differences in decapsulized lenses: the CPM in the SPARC-null lenses were 1.6 times (1-month-old) and 2.5 times (3-month-old) the values of the corresponding $^{+/+}$ lenses (data not shown). The conclusions are that (1) SPARC-null lens capsules exhibited increased levels of dye or [^{3}H]-thymidine, but not ^3H_2O, (2) SPARC-null lens capsules and underlying cells allowed more dye, [^{3}H]-thymidine or ^3H_2O to penetrate into the lens mass and (3) the amount of ^3H_2O or [^{3}H]-thymidine that penetrated into the lenses was substantially greater in 3-month-old mice than in 1-month-old mice. The normal lens capsule is permeable to water and small macromolecules (Newell, 1996). Trypan blue dye and [^{3}H]-thymidine can diffuse through the capsule and enter the lens mass (Fig. 10). However, within 20 minutes, SPARC-null lenses contained more dye and radioactive tracer relative to wt controls, a result indicating that disorganization of the capsule and its underlying cells is associated with increased penetration into SPARC-null lenses. The dye was distributed mostly in the equatorial region (Fig. 10A, arrowheads). This observation is consistent with our data from EM and immunostaining of collagen type IV, that is, the lens capsule posterior to the bow region is the initial and principal site of the structural abnormality (Figs 8 and 9).

Wt and SPARC-null lenses had similar wet weights (4.09 mg versus 4.15 mg, respectively) (Fig. 11). After dehydration, the dry weight of 1-month-old SPARC-null lenses was less than that of the SPARC$^{+/+}$ lenses (0.77 mg versus 1.13 mg, respectively), owing to the increased water content of the SPARC-null lenses. This observation is consistent with the ^3H_2O penetration assay. Breakdown of the physiological barrier of SPARC-null lenses would allow increased water flux and/or altered ion transport, which would perturb the osmotic balance in the lens fiber cell membranes and lead to the swelling of fibers. The results are the first to indicate the possible influence of SPARC on permeability in the lens.

Discussion

In this study, we show that cataract formation in SPARC-null mice is associated with compromised structure of the lens capsule as early as 1 month of age. Our results demonstrate that SPARC is a key protein affecting the maintenance and structural integrity of the lens capsule and the regulation of normal cellular homeostasis in lens cells.

The lens capsule is a specialized ECM that (a) is responsible for lens compartmentalization and the maintenance of lens tissue structure, (b) controls fluid and substrate exchange and (c) regulates normal lens epithelial cell growth and differentiation. In the SPARC-null lens, we propose that its compromised lens capsule contributes to altered homeostasis

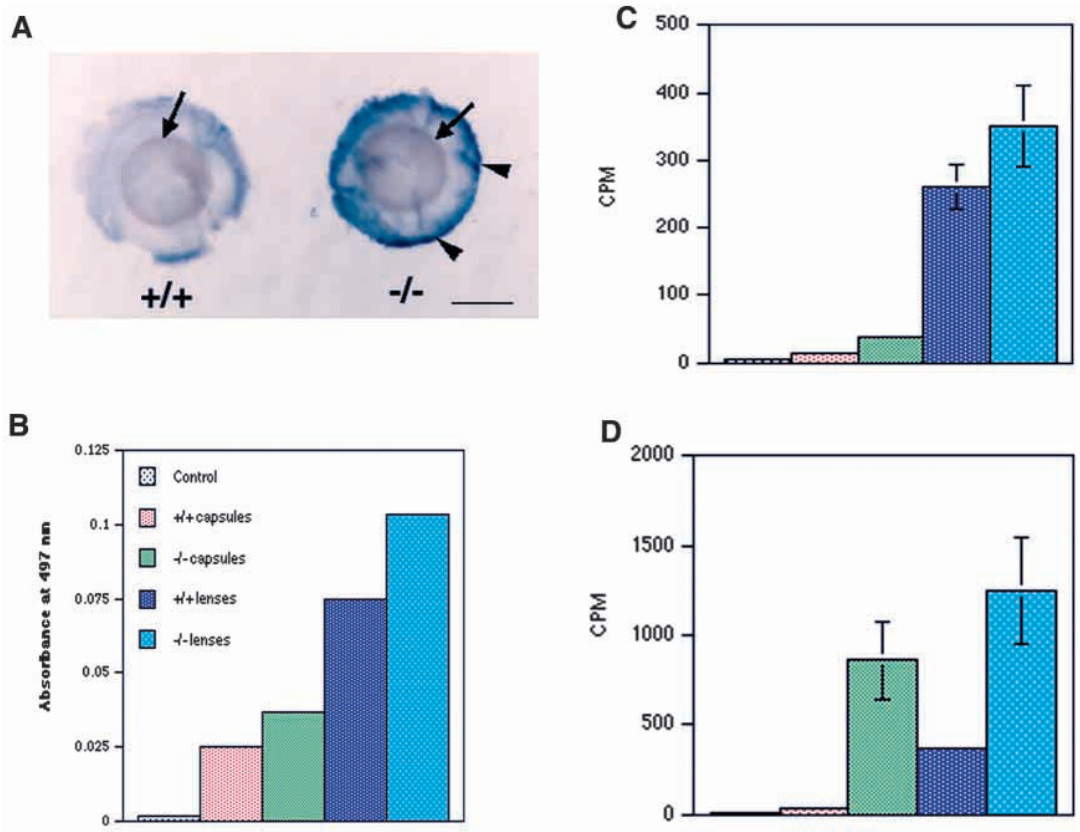

Fig. 10. SPARC-null lenses exhibit increased penetration of dye and radioactive tracer. (A) A pair of intact lenses [1 month old, SPARC$^{+/+}$ versus SPARC-null ($^{-/-}$)] was immersed in trypan blue dye for 20 minutes, as described in the Materials and Methods. Lens capsules with attached epithelium were removed, and decapsulized lenses were immediately photographed. The penetration of the blue dye was increased in the $^{-/-}$ lens (equator and peripheral cortex, arrowheads), relative to the $^{+/+}$ lens. Arrows indicate the nucleus of each lens. Bar, 460 μm. (B) Quantitative analysis of dye penetrating into the lens fiber cells and of dye absorbed in the lens capsules with attached epithelial cells. Control (PBS) absorbance was less than 0.005 nm. The amount of dye bound within the capsules of the $^{-/-}$ lenses was 1.46 times the value of that bound within the $^{+/+}$ lenses. The dye content measured in decapsulized lenses was 1.38 times the value of $^{+/+}$ lenses. A representative experiment with six lenses for each group (1 month old) is shown. (C,D) Quantitative analysis of [^{3}H]-thymidine tracer penetration into the capsules (with epithelium attached) and lenses in 1-month-old (C) and 3-month-old mice (D). The [^{3}H]-thymidine CPM within the capsules of the $^{-/-}$ lenses were 2.5 times (1 month old) and 27 times (3 month old) the values of the corresponding $^{+/+}$ lens capsules. The [^{3}H]-thymidine CPM in $^{-/-}$ decapsulized lenses were 1.34 times (1 month old) and 3.35 times (3 month old) the values of the corresponding $^{+/+}$ lenses. Bars in C and D are identified as shown in B. CPM, counts per minute; White bar, control; pink bar and green bar, $^{+/+}$ and $^{-/-}$ capsules with attached lens epithelial cells, respectively; purple bar and light blue bar, $^{+/+}$ and $^{-/-}$ decapsulized lenses, respectively. Data are the means±s.d.

of the lens and the transport of water, small molecules and ions into the strictly aligned lens fiber cells. The result of these changes is swelling of the lens fibers, one of the hallmarks of an osmotic cataract (e.g., the diabetic cataract) (Bond et al., 1996).

SPARC-null mice begin to exhibit cortical alterations at 1 month of age, when secondary lens fibers located in the cortex and bow region appear swollen (Fig. 3). Ultrastructural analysis disclosed defects in the lens capsule and lens cells in 1 month-old SPARC-null mice (Fig. 8A,B). At this early stage, the capsule structure begins to appear disorganized. The structurally compromised lens capsule, especially at the equator, was associated with increased dye and radioactive tracer penetration into the lens (Fig. 10A, arrowheads; Fig. 10), a result indicating that SPARC-null lenses lack normal permeability. The swelling of the fibers in the bow region at early stages (Fig. 3F), and the numerous vacuoles in this region, could be caused by the disruption of normal cell-matrix interactions and initial damage of the posterior lens capsule behind the equator in the SPARC-null lens. The progression of the fiber cell protrusion into the

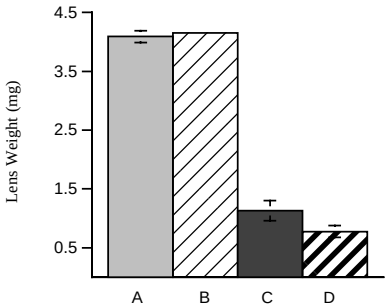

Fig. 11. Water content in wt and SPARC-null lenses. Lenses of 1 month-old mice were weighed immediately after dissection. After dehydration at 90°C for 16 hours, the dry weight of each lens was determined. (A,B) SPARC$^{+/+}$ and SPARC-null ($^{-/-}$) lenses, respectively, prior to dehydration. (C,D) $^{+/+}$ and $^{-/-}$ lenses, respectively, after dehydration. The $^{+/+}$ lenses lose 72% of their weight upon drying, whereas $^{-/-}$ lenses lose 81% of their weight upon drying. Values are derived from six lenses per group.

lens capsule is consistent with the amounts of tracers in the lens capsules and lenses (Fig. 10, 1 month versus 3 months). The presence of defective lens capsular structure is consistent with the swelling of the fiber cells.

The lens capsule is an unusally thick basement membrane. Its intrinsic structural components include collagen type IV, laminin, nidogen and heparan sulfate proteoglycan (Cammarata et al., 1986; Starkey et al., 1987; Inoue, 1994). The importance of heparan sulfate proteoglycan in the BM for regulation of the permeability of ions and macromolecules has been amply demonstrated in the glomerular BM (Groffen et al., 1999). Heparan sulfate glycosaminoglycans provide a charge-sieving function, as the polyanionic chains are associated with large spheres of hydration, which would leave little free water between macromolecules. Since the distribution of glycosaminoglycans appears to be different in normal and cataractous lens capsules (Winkler et al., 2001), it is likely that alteration of anionic sites would affect the permeability of metabolites as well. Disruption of the organization of the macromolecular network of the lens BM is predicted to facilitate the passage of water, ions and possibly larger molecules across the BM. In fact, increased permeability of the lens capsule to water was found in lenses with cortical cuneiform opacities, to the exclusion of the nucleus (Fisher, 1977; Maraini and Mangili, 1973). Moreover, such increases were also found in the rabbit lens, in which the posterior lens capsule had been perforated, and in adult human diabetic lenses, in which edematous lens fibers were correlated with a 37% increase in hydraulic permeability (Fisher, 1985; Fisher and Wakely, 1976). SPARC-null lenses with cortical opacity exhibited an increased permeability to water, relative to wt lenses, that could disrupt the Na^+ and K^+ balance in fiber cells and could cause cell swelling. Dysfunctional cell membrane selectivity to ions has been correlated with increased permeability (Duncan and Croghan, 1969) and is consistent with the association of increases in ion flux with the onset of cataracts (Matsuda et al., 1982). The SPARC-null mice that exhibit cortical lenticular opacity, but no involvement of the nucleus until later stages, might represent another cataract model with compromised permeability of the lens capsule.

Lens capsular BM is not a static structure but is continually produced and remodeled by lens epithelial cells and newly differentiated fiber cells (Johnson and Beebe, 1984). As SPARC regulates the production of ECM proteins and binds to several collagens including the BM collagen type IV (Lane et al., 1992; Kamihagi et al., 1994; Francki et al., 1999; Sage et al., 1989; Sasaki et al., 1998), our data are consistent with the proposal that SPARC affects the organization and assembly of the components in the lens capsular matrix. Additionally, it is highly likely that lens cells without SPARC exhibit abnormal behavior. For example, the protrusion of the fiber cells into the lens capsule indicates not only that the lens capsule is abnormal, but also that these fiber cells without SPARC become invasive and pathological. SPARC-null lens cells are clearly altered in their morphology, but their production of ECM components and heparan sulfate proteoglycan, as well as their migratory, adhesive and differentiation properties, are poorly understood and are currently under investigation in our laboratory.

It is interesting that age-related changes in the lenses of Fischer rats were morphologically similar to those observed in SPARC-null lenses. For example, at 18 months of age, Uga et al. observed posterior invasion by the processes of the cortical fiber cells toward the lens capsule and swelling of anterior cortical fibers (Uga et al., 1996). Whether or not age-related human cataracts exhibit any abnormalities in the lens capsule, as seen in SPARC-null mice, has not been reported and needs to be investigated. Elevated levels of SPARC mRNA and protein have been described in human cataratous lenses, an observation leading Kantorow et al. to propose that SPARC is associated with the process of human cataractogenesis (Kantorow et al., 2000).

In this study, the labeling of proliferating cells in vivo with BrdU tested the hypothesis that accelerated proliferation of lens epithelial cells in SPARC-null mice results in lenticular opacity. No significant differences in cell proliferation between wt and SPARC-null lenses from E14 to 4 months of age were observed, despite the presence of opacity in SPARC-null mice that began at 1 month. Only in SPARC-null mice of 7 months and older was there a significant increase in BrdU-positive cells, which occurred concomitantly with inflammation and tissue necrosis in the ruptured posterior area (Norose et al., 1998). It is likely that inflammatory cells recruited into the injured area stimulated an increase in BrdU-positive cells by their release of cytokines. In addition, there were no significant changes in the levels of MIP 26 and crystallins in SPARC-null lenses. The absence of proteolytic degradation of crystallins and MIP 26 indicate that the activation of proteases for these substrates is minimal in the SPARC-null lenses, at least up to 3 months of age.

SPARC interacts with ECM proteins (e.g., collagen types I, III and IV) and growth factors (platelet-derived growth factor and vascular endothelial growth factor) and influences cell proliferation, migration, adhesion, differentiation and/or barrier function (Yan and Sage, 1999; Brekken and Sage, 2001; Goldblum et al., 1994). If SPARC is important in the regulation of the distribution and organization of lens BM matrix, it follows that this protein is likely to influence lens epithelial cell growth and differentiation. This study indicates that compromised lens permeability is a consequence of the absence of SPARC and contributes to cataract formation. The mechanism(s) that accounts for the defects in the lens capsule will contribute to our understanding of the biological functions of SPARC and the organization of lens BM proteins and proteoglycans, as well as the importance of cell-matrix interactions in the lens.

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References

Bassuk, J. A., Birkebak, T., Rothmier, J. D., Clark, J. M., Bradshaw, A., Muchowski, P. J., Howe, C. C., Clark, J. I. and Sage, E. H. (1999). Disruption of the Sparc locus in mice alters the differentiation of lenticular epithelial cells and leads to cataract formation. *Exp. Eye Res.* **68**, 321-331.

Benedetti, E. L., Dunia, I. and Bloemendal, H. (1974). Development of junctions during differentiation of lens fibers. *Proc. Natl. Acad. Sci. USA* **71** 5073-5077.

Bond, J., Green, C., Donaldson, P. and Kistler, J. (1996). Liquefaction of

cortical tissue in diabetic and galactosemic rat lenses defined by confocal laser scanning microscopy. *Invest. Ophthalmol. Vis. Sci.* **37**, 1557-1565.
Bornstein, P. (1995). Diversity of function is inherent in matricellular proteins: an appraisal of thrombospondin 1. *J. Cell Biol.* **130**, 503-506.
Bosman, F. T., Cleutjens, J., Beek, C. and Havenith, M. (1989). Basement membrane heterogeneity. *Histochem. J.* **21**, 629-633.
Brekken, R. A. and Sage, E. H. (2001). SPARC, a matricellular protein: at the crossroads of cell-matrix communication. *Matrix Biol.* **19**, 816-827.
Cammarata, P. R., Cantu-Crouch, D., Oakford, L. and Morrill, A. (1986). Macromolecular organization of bovine lens capsule. *Tissue Cell* **18**, 83-97.
Duncan, G. and Croghan, P. C. (1969). Mechanisms for the regulation of cell volume with particular reference to the lens. *Exp. Eye Res.* **8**, 421-428.
Fisher, R. F. (1977). Changes in the permeability of the lens capsule in senile cataract. *Trans. Ophthalmol. Soc. UK* **97**, 100-103.
Fisher, R. F. (1985). The structure and function of basement membrane (lens capsule) in relation to diabetes and cataract. *Trans. Ophthalmol. Soc. UK* **104**, 755-759.
Fisher, R. F. and Wakely, J. (1976). Changes in lens fibres after damage to the lens capsule. *Trans Ophthalmol Soc UK* **96**, 278-284.
Fitch, J. M., Mayne, R. and Linsenmayer, T. F. (1983) Developmental acquisition of basement membrane heterogeneity: type IV collagen in the avian lens capsule. *J. Cell Biol.* **97**, 940-943.
Francki, A., Bradshaw, A. D., Bassuk, J. A., Howe, C. C., Couser, W. G. and Sage, E. H. (1999). SPARC regulates the expression of collagen type I and transforming growth factor-beta1 in mesangial cells. *J. Biol. Chem.* **274**, 32145-32152.
Gilmour, D. T., Lyon, G. J., Carlton, M. B., Sanes, J. R., Cunningham, M. J., Anderson, J. R., Hogan, B. L., Evans, M. J. and Colledge, W. H. (1998). Mice deficient for the secreted glycoprotein SPARC/osteonectin/BM40 develop normally but show severe age-onset cataract formation and disruption of the lens. *EMBO J.* **17**, 1860-1870.
Goldblum, S. E., Ding, X., Funk, S. E. and Sage, E. H. (1994). SPARC (secreted protein acidic and rich in cysteine) regulates endothelial cell shape and barrier function. *Proc. Natl. Acad. Sci. USA* **91**, 3448-3452.
Groffen, A. J., Veerkamp, J. H., Monnens, L. A. and van den Heuvel, L. P. (1999). Recent insights into the structure and functions of heparan sulfate proteoglycans in the human glomerular basement membrane. *Nephrol. Dial. Transplant.* **14**, 2119-2129.
Inoue, S. (1994). Basic structure of basement membranes is a fine network of "cords", irregular anastomosing strands. *Microsc. Res. Tech.* **28**, 29-47.
Johnson, M. C. and Beebe, D. C. (1984). Growth, synthesis and regional specialization of the embryonic chicken lens capsule. *Exp. Eye Res.* **38**, 579-592.
Kamihagi, K., Katayama, M., Ouchi, R. and Kato, I. (1994). Osteonectin/SPARC regulates cellular secretion rates of fibronectin and laminin extracellular matrix proteins. *Biochem. Biophys. Res. Commun.* **200**, 423-428.
Kantorow, M., Huang, Q., Yang, X. J., Sage, E. H., Magabo, K. S., Miller, K. M. and Horwitz, J. (2000). Increased expression of osteonectin/SPARC mRNA and protein in age-related human cataracts and spatial expression in the normal human lens. *Mol. Vis.* **6**, 24-29.
Kupprion, C., Motamed, K. and Sage, E. H. (1998). SPARC (BM-40, osteonectin) inhibits the mitogenic effect of vascular endothelial growth factor on microvascular endothelial cells. *J. Biol. Chem.* **273**, 29635-29640.
Lane, T. F. and Sage, E. H. (1990). Functional mapping of SPARC: peptides from two distinct Ca^{++}-binding sites modulate cell shape. *J. Cell Biol.* **111**, 3065-3076.
Lane, T. F., Iruela-Arispe, M. L. and Sage, E. H. (1992). Regulation of gene expression by SPARC during angiogenesis in vitro. Changes in fibronectin, thrombospondin-1, and plasminogen activator inhibitor-1. *J. Biol. Chem.* **267**, 16736-16745.
Lane, T. F. and Sage, E. H. (1994). The biology of SPARC, a protein that modulates cell-matrix interactions. *FASEB J.* **8**, 163-173.
Lee, S. M., Lin, S. Y., Li, M. J. and Liang, R. C. (1997). Possible mechanism of exacerbating cataract formation in cataractous human lens capsules induced by systemic hypertension or glaucoma. *Ophthalmic Res.* **29**, 83-90.
Li, Y., Yan, Q. and Wolf, N. S. (1997). Long-term caloric restriction delays age-related decline in proliferation capacity of murine lens epithelial cells in vitro and in vivo. *Invest. Ophthalmol. Vis. Sci.* **38**, 100-107.
Maraini, G. and Mangili, R. (1973). Differences in proteins and in the water balance of the lens in nuclear and cortical types of senile cataract. In *The Human Lens in Relation to Cataract* (K. M. Elliott and D. W. Fitzsimmons, eds), pp. 79-97. Amsterdam: Elsevier Excerpta Medica.
Matsuda, H., Giblin, F. J. and Reddy, V. N. (1982). The effect of X-irradiation on Na-K ATPase and cation distribution in rabbit lens. *Invest. Ophthalmol. Vis Sci.* **22**, 180-185.
Mayer, U., Aumailley, M., Mann, K., Timpl, R. and Engel, J. (1991). Calcium-dependent binding of basement membrane protein BM-40 (osteonectin, SPARC) to basement membrane collagen type IV. *Eur. J. Biochem.* **198**, 141-150.
Maurer, P., Hohenadl, C., Hohenester, E., Göhring, W., Timpl, R, and Engel, J. (1995). The C-terminal portion of BM-40 (SPARC/osteonectin) is an autonomously-folding and crystallisable domain that binds calcium and collagen IV. *J. Mol. Biol.* **253**, 347-357.
Murphy-Ullrich, J. E., Lane, T. F., Pallero, M. A. and Sage, E. H. (1995). SPARC mediates focal adhesion disassembly in endothelial cells through a follistatin-like region and the calcium-binding EF-hand. *J. Cell Biochem.* **57**, 341-350.
Newell, F. W. (1996). Physiology and biochemistry of the eye. In *Ophthalmology principles and concepts* (K. Kirst, ed.), pp. 74-102. St Louis, MO: Mosby Year Book Inc.
Norose, K., Clark, J. I., Syed, N. A., Basu, A., Heber-Katz, E., Sage, E. H. and Howe, C. C. (1998). SPARC deficiency leads to early-onset cataractogenesis. *Invest. Ophthalmol. Vis. Sci.* **39**, 2674-2680.
Norose, K., Lo, W. K., Clark, J. I., Sage, E. H. and Howe, C. C. (2000). Lenses of SPARC-null mice exhibit an abnormal cell surface-basement membrane interface. *Exp. Eye Res.* **71**, 295-307.
Raines, E. W., Lane, T. F., Iruela-Arispe, M. L., Ross, R. and Sage, E. H. (1992). The extracellular glycoprotein SPARC interacts with platelet-derived growth factor (PDGF)-AB and -BB and inhibits the binding of PDGF to its receptors. *Proc. Natl. Acad. Sci. USA* **89**, 1281-1285.
Sage, H., Vernon, R. B, Funk, S. E., Everitt, E. A. and Angello, J. (1989). SPARC, a secreted protein associated with cellular proliferation, inhibits cell spreading in vitro and exhibits Ca+2-dependent binding to the extracellular matrix. *J. Cell Biol.* **109**, 341-356.
Sage, E. H. (1997). Terms of attachment: SPARC and tumorigenesis. *Nat. Med.* **3**, 144-146.
Sage. E. H. and Bornstein, P. (1991). Extracellular proteins that modulate cell-matrix interactions. SPARC, tenascin, and thrombospondin. *J. Biol. Chem.* **266**, 14831-14834.
Sasaki, T., Hohenester, E., Göhring, W. and Timpl, R. (1998). Crystal structure and mapping by site-directed mutagenesis of the collagen-binding epitope of an activated form of BM-40/SPARC/osteonectin. *EMBO J.* **17**, 1625-1634.
Shiels, A. and Bassnett, S. (1996). Mutations in the founder of the MIP gene family underlie cataract development in the mouse. *Nat. Genet.* **12**, 212-215.
Starkey, J. R., Stanford, D. R., Magnuson, J. A., Hamner, S., Robertson, N. P. and Gasic, G. J. (1987). Comparison of basement membrane matrix degradation by purified proteases and by metastatic tumor cells. *J. Cell. Biochem.* **35**, 31-49.
Timpl, R. and Dziadek, M. (1986). Structure, development, and molecular pathology of basement membranes. *Int. Rev. Exp. Pathol.* **29**, 1-112.
Tremble, P. M., Lane, T. F., Sage, E. H. and Werb, Z. (1993). SPARC, a secreted protein associated with morphogenesis and tissue remodeling, induces expression of metalloproteinases in fibroblasts through a novel extracellular matrix-dependent pathway. *J. Cell Biol.* **121**, 1433-1444.
Uga, S., Obara, Y., Takehana, M., Nishigori, H., Hikida, M. and Mibu, H. (1996). Morphological study of age-related changes in Fischer rat lens. *Jpn. J. Ophthalmol.* **40**, 33-41.
Wight, T. N., Lara, S., Riessen, R., le Baron, R. and Isner, J. (1997). Selective deposits of versican in the extracellular matrix of restenotic lesions from human peripheral arteries. *Am. J. Pathol.* **151**, 963-973.
Winkler, J., Wirbelauer, C., Frank, V. and Laqua, H. (2001). Quantitative distribution of glycosaminoglycans in young and senile (cataractous) anterior lens capsules. *Exp. Eye Res.* **72**, 311-318.
Wride, M. A. (1996). Cellular and molecular features of lens differentiation: a review of recent advances. *Differentiation* **61**, 77-93.
Yan, Q., Sage, E. H. and Hendrickson, A. E. (1998). SPARC is expressed by ganglion cells and astrocytes in bovine retina. *J. Histochem. Cytochem.* **46**, 3-10.
Yan, Q. and Sage, E. H. (1999). SPARC, a matricellular glycoprotein with important biological functions. *J. Histochem. Cytochem.* **47**, 495-506.
Yan, Q., Clark, J. I. and Sage, E. H. (2000). Expression and characterization of SPARC in human lens and in the aqueous and vitreous humors. *Exp. Eye Res.* **71**, 81-90.
Yurchenco, P. D. and Schittny, J. C. (1990). Molecular architecture of basement membranes. *FASEB J.* **4**, 1577-1590.