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ARTICLE

Expression of the Matricellular Protein SPARC in Murine Lens: SPARC Is Necessary for the Structural Integrity of the Capsular Basement Membrane

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SUMMARY SPARC (Secreted Protein, Acidic and Rich in Cysteine) is a matricellular glycoprotein that modulates cell proliferation, adhesion, migration, and extracellular matrix (ECM) production. Although SPARC is generally abundant in embryonic tissues and is diminished in adults, we have found that the expression of SPARC in murine lens persists throughout embryogenesis and adulthood. Our previous studies showed that targeted ablation of the SPARC gene in mice results in cataract formation, a pathology attributed partially to an abnormal lens capsule. Here we provide evidence that SPARC is not a structural component of the lens capsule. In contrast, SPARC is abundant in lens epithelial cells, and newly differentiated fiber cells, with stable expression in wild-type mice up to 2 years of age. Pertubation of the lens capsule in animals lacking SPARC appears to be a consequence of the invasion of the lens cells situated beneath the capsule. Immunoreactivity for SPARC in the lens cells was uneven, with minimal reactivity in the epithelial cells immediately anterior to the equator. These epithelial cells appeared essentially noninvasive in SPARC-null mice, in comparison to the centrally located anterior epithelial cells, in which strong labeling by anti-SPARC IgG was observed. The posterior lens fibers exhibited cytoplasmic extensions into the posterior lens capsule, which was severely damaged in SPARC-null lenses. The expression of SPARC in wild-type lens cells, together with the abnormal lens capsule in SPARC-null mice, indicated that the structural integrity of the lens capsule is dependent on the matricellular protein SPARC. The effects of SPARC in the lens appear to involve regulation of lens epithelial and fiber cell morphology and functions rather than deposition as a structural component of the lens capsule. **(J Histochem Cytochem 51:503–511, 2003)**

KEY WORDS
SPARC
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lens fiber cells
collagen type IV
β_1 integrin
matricellular protein

THREE rather unique components distinguish the mammalian ocular lens: (a) the lens capsule, a thick basement membrane and specialized extracellular matrix (ECM) that regulates lens cell growth and differentiation; (b) epithelial cells, which form a monolayer underneath the anterior capsule; and (c) differentiated transparent fiber cells that constitute the lens mass (Figure 1 A). During the development of the lens, epithelial cells exit from the cell cycle, migrate to the post-equatorial region, and undergo substantial morphological and biochemical changes to form concentric layers of transparent fiber cells (Kuzsak et al. 1999). In the process of lens fiber cell formation, the ECM proteins in the capsule interact directly with the basal surface of the lens epithelial cells to influence their migration, adhesion, change in shape, and maturation (Menko et al. 1998; Blakely et al. 2000). This interaction influences lens development and differentiation through mechanisms that are not clearly understood. The matricellular protein SPARC (Secreted Protein, Acidic and Rich in Cysteine) appears to function as a modulator of ECM production and cell–ECM interactions in certain tissues, and possibly, in the mor-

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phogenesis and/or maintenance of the lens capsule (Bornstein and Sage 2002; Yan et al. 2002). Targeted disruption of the sparc gene in mice results in cataract formation (Gilmour et al. 1998; Norose et al. 1998). We have reported that an abnormal lens capsule contributes importantly to SPARC-null cataractogenesis (Yan et al. 2002). To understand the role of SPARC in the pathology of lens basement membrane in SPARC-null mice, we have addressed the following questions in this report: (a) when does expression of SPARC in the lens occur; (b) is SPARC a structural component of the lens capsule; (c) what causes the disruption of the lens capsule in SPARC-null mice; and (d) is the location of SPARC in the lens consistent with the abnormal capsules in SPARC-null lenses?

SPARC is a secreted, Ca^2+-binding glycoprotein that interacts with a variety of ECM proteins and growth factors (reviewed in Yan and Sage 1999; Brekken and Sage 2001). First identified as a major noncollagenous protein of bone (termed osteonectin; Termine et al. 1981) and also purified from a basement membrane tumor (termed BM-40; Dziadek et al. 1986), SPARC is produced in most embryonic tissues but its distribution is rather limited in the adult (Holland et al. 1987; Sage et al. 1989). In vivo, SPARC is associated with activities such as cell proliferation, migration, matrix remodeling, and morphogenesis, consistent with its functions identified in vitro, e.g., deadhesion and anti-proliferation (Bornstein and Sage 2002). Despite several activities demonstrated by studies in vitro, the function(s) of SPARC in vivo remains unclear. Our recent study with SPARC-null mice demonstrated that SPARC participates significantly in the maintenance of the lens capsule. In the absence of SPARC, the structural integrity of the capsule was aberrant as early as 1 month, a condition leading to subsequent rupture of the posterior lens capsule at 5–8 months (Yan et al. 2002). To understand how SPARC influences lens capsular structure and lens cell behavior, we first investigated the developmental expression of SPARC in the lens. We provide evidence that SPARC is produced in embryonic lenses as early as E12. SPARC is not a structural element of the lens capsule. It is abundant in the lens epithelial cells and in newly differentiated fiber cells, although its distribution among these cells appears to be distinct. We observed that the special areas of capsular damage in the SPARC-null lens correspond closely with cells in which the expression of SPARC would normally be highest. Finally, we show that the disrupted areas in the SPARC-null capsule corresponded with the location of invadopodia of the lens cells. This study indicates that SPARC is critical for maintenance of the function of the lens cells that produce the capsule but that it does not act primarily as a structural component of the lens basement membrane.

Materials and Methods

Animals

129SvEv × C57BL/6j wild-type (wt, +/+) and SPARC-null (−/−) mice were maintained in a pathogen-free facility. The treatment and use of the mice in this study conformed to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmology and Vision Research.

Immunohistochemistry

Eyeballs from mice (E12 to 18 months of age) were removed and were fixed by immersion in methyl Carnoy solution (60% methanol, 30% chloroform, and 10% glacial acidic acid) for 4 hr at room temperature (RT). The eyeballs were dehydrated in a series of ethanol solutions (70%, 90%, and 100%) and were embedded in paraffin.

Serial 5-μm sections were cut, deparaffinized, rehydrated, and washed in PBS. Auto/Zyme was used for unmasking of the tissue sections (Biomeda; Foster City, CA). Nonspecific binding sites were blocked by incubation in 20% Aqua serum (Biomeda) in PBS with 0.05% Tween-20 for 2 hr at RT. Sections were incubated for 2 hr with agitation at RT with the following antibodies: polyclonal goat anti-mouse SPARC IgG; polyclonal rabbit anti-mouse SPARC IgG (R & D Systems, Minneapolis, MN; 10 μg/ml); rabbit anti-mouse laminin-1 IgG (Sigma, St Louis, MO; 5 μg/ml); rabbit anti-mouse collagen type IV (Chemicon International, Temecula, CA; 5 μg/ml); rat anti-mouse β_1-integrin (Chemicon International; 5 μg/ml). Negative controls included replacement of primary antibodies by normal mouse IgG or PBS, or primary antibody applied to SPARC-null lens sections. Sections were washed three times for 10 min each in PBS, and were incubated in fluorescein isothiocyanate (FITC)- or rhodamine-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories; West Grove, PA).

Immunoblotting

Lens capsules were removed from the cell mass of the lens under a dissecting microscope. The lens epithelium was mechanically scraped off the capsule. The capsule was immersed in cold distilled water for 20 min to lyse any remaining epithelial cells and was washed thoroughly in PBS. The capsules were briefly immersed in Hoechst 33258 fluorochrome (0.1 μg/ml in distilled water; Flow Laboratories, McLean, VA) and were examined under fluorescence microscopy to ensure the complete removal of attached cells. Capsules were homogenized and were solubilized in 40 μl Laemmli's SDS-PAGE sample buffer containing 10 mM dithiothreitol, heated for 5 min at 100C, and analyzed by SDS-PAGE on 10% polyacrylamide minigels (2 capsules per lane). Standard immunoblotting procedure was followed (Yan et al. 2000) and the blots were exposed to polyclonal anti-mouse SPARC IgG. The same blot was stripped and reprobed with antibody against glyceraldehyde phosphate dehydrogenase (GAPDH) to verify that there was no contamination of the lens basement membrane samples with lens epithelial cell protein.

RT-PCR

Total RNA was isolated from lens epithelial cells and lens fiber cells of wt and SPARC-null mice with a RNeasy Mini Kit (Qiagen; Valencia, CA). Lens fiber cells initially extracted in chloroform and isopropanol were homogenized in TRI reagent (Molecular Research Center; Cincinnati, OH). 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 a reverse transcription kit (Omniscript RT kit; Qiagen). Equal amounts of RNA were transcribed from each preparation. cDNA was amplified using the following primer pairs: SPARC, ATGAGGGCCTGGATCTTCTTTC/GGAAGAGTCGAAGGTCTTGTTGTC; CX43, GTTCAGCCTGAGTGCGGTCTAC/TTCCCTTCACGCGATCCTT; and GAPDH, GACCCCTTCATTGACCTCAACT/ACCAAGTGTGGGGTAGTGTTTG. The PCR program was 12 min at 94C, followed by 35 cycles of 45 sec at 95C, 59 sec at 65C, 2 min at 72C, followed by a final extension of 8 min at 72C. Products were separated on agarose gels and were visualized by staining with ethidium bromide.

Results

Expression and Distribution of SPARC in Embryonic and Adult Lenses

The distribution of SPARC protein in the lens at three embryonic stages (E11.5, E13.5, and E17.5) and in the adult up to 18 months of age is shown in Figure 1. Reactivity with anti-SPARC IgG was first detected in the primary lens fiber cells at E11.5 (Figure 1B, arrow) and E13.5 (Figure 1D, arrow). At E17.5 (Figure 1F), SPARC was prominent in the anterior lens epithelium (single long arrow), and was diminished before the bow region (arrowhead). Behind the bow region, SPARC was evident at the basal surface of the lens fibers where they contact the posterior capsule (double long arrows). Secondary fibers (E17.5) appear essentially devoid of SPARC (short small arrows), although the lens nucleus (primary fibers) was weakly reactive with anti-SPARC IgG (*). Immunostaining in the anterior lens epithelium remained similar in both intensity and distribution pattern at postnatal day 5 (Figure 1H), day 13 (Figure 1I), 1 month (Figure 1J), 4 months (Figure 1K), and 18 months (Figure 1L), whereas the intensity of the stain in the primary fibers became insignificant (data not shown). The lens epithelial cells flatten, and the capsule thickens, after 1 month of age, but the intensity of the staining for SPARC was not decreased in the epithelium up to 18 months of age (Figures 1J–1L). Importantly, the lens capsule was negative in all the ages examined (Figures 1J–1L, arrowheads). SPARC-null lenses (Figures 1E and 1G) exposed to the same conditions as wt lenses (Figures 1D and 1F), and wt lens (E11.5) without primary antibody (Figure 1C), showed no immunoreactivity.

Two capsules (approximately 70 μg protein) were also examined for the presence of SPARC. By immunoblotting analysis, the levels of SPARC appeared similar in lenses from 1 month to 2 years of age (Figure 1M). Moreover, proteolytic cleavage of SPARC was not observed in the lenses, although the polyclonal antibody recognizes internal sequences of SPARC (Yan et al. 2000).

SPARC Is Not Detected in Murine Lens Basement Membrane

The murine lens capsule exhibited no immunoreactivity with anti-SPARC IgG (Figure 1), consistent with our observations in human (Yan et al. 2000) and bovine lenses (Yan and Sage 1999). Explanations for the apparent absence of SPARC include the following: (a) the epitope is masked by other components in the basement membrane; (b) the three-dimensional structure of the antigen in the network of the capsular components precludes the exposure of its epitope(s) to the antibody; or (c) SPARC is degraded in the capsule (Yan and Sage 1999). In addition to unmasking procedures, we re-examined this question biochemically (Figure 2). The complete removal of the lens epithelial cells attached to the capsules was first confirmed by the absence of nuclear labeling by Hoechst 33258 dye under fluorescence microscopy (not shown), and was further verified by the absence of GAPDH after immunoblotting of the various fractions (Figure 2). It is critical to minimize contamination of the lens capsule with epithelium because the cells contain abundant SPARC. Our data indicate that lens capsules without epithelial cells do not contain SPARC (Figure 2, Lane 2). Moreover, the SPARC present within or associated with the cells exhibited no degradation.

Cellular Localization of SPARC in Lens Epithelial Cells and Newly Differentiated Fiber Cells

The presence of SPARC in the lens was examined further in animals 1 month of age (Figure 3). The cytoplasm of the anterior lens epithelial cells was reactive with anti-SPARC IgG (Figure 3A, arrow, inset), whereas the cell nucleus was not (Figure 3A, arrowhead, inset). SPARC-null lens epithelial cells showed no reaction with the antibody (Figure 3B, arrow). The area showing the least amount of SPARC in the lens epithelial cells was in the region anterior to the equator (Figure 3C, between the arrows). Newly differentiated lens fiber cells exhibited immunoreactivity with anti-SPARC IgG, particularly at the basal surface near the contacts with the capsule (Figures 3D–3F represent a continuous series of photographs toward the posterior pole). The lens capsule was not stained with anti-SPARC IgG in either the anterior (Figures 3A and 3C) or posterior region (Figures 3D–3F).

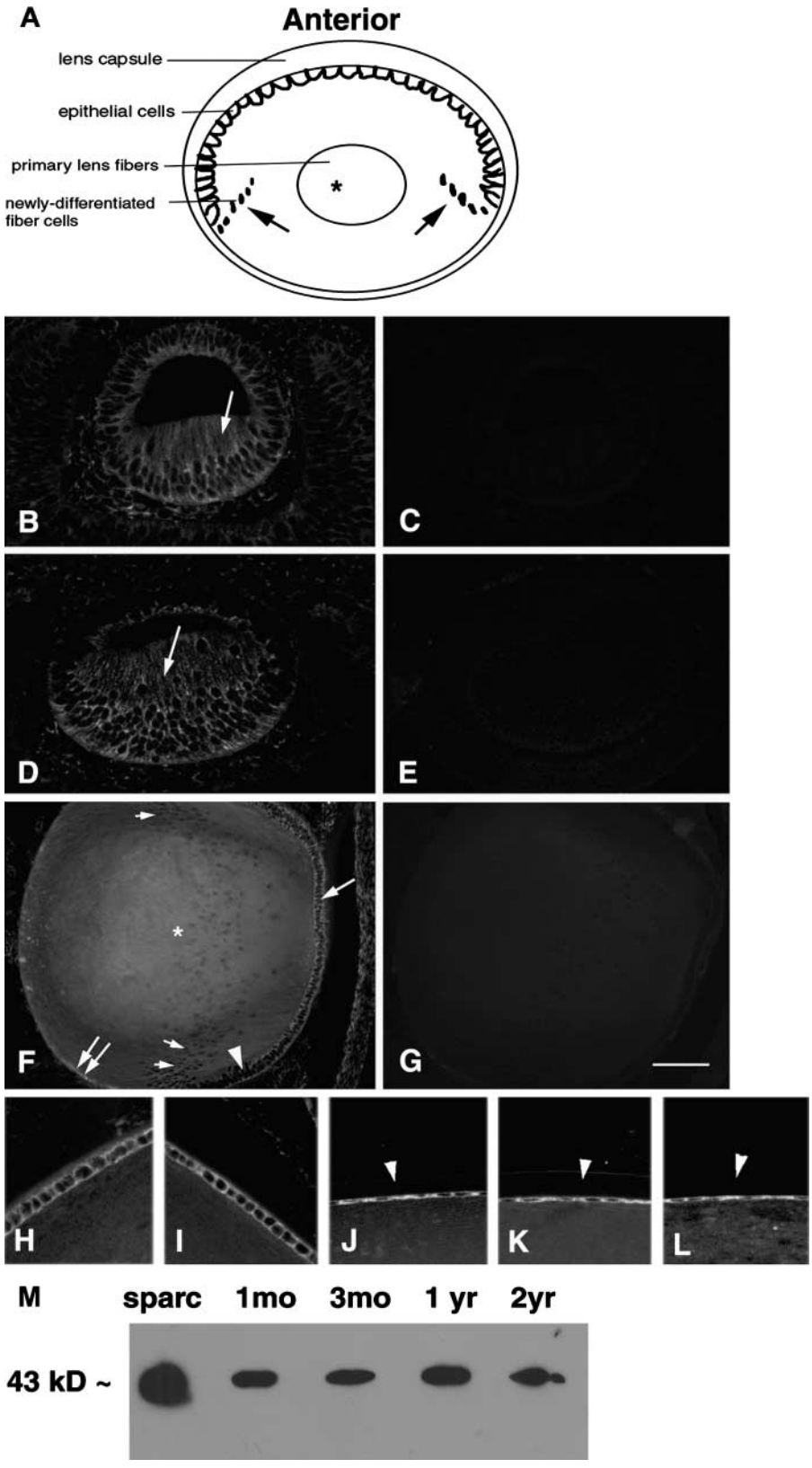

Figure 1 Distribution of SPARC in E12 to 18 months of age. (**A**) Schematic of an adult mouse lens (lens components are not drawn to scale). The lens capsule envelops the entire lens but is considerably thicker anteriorly, as shown. The capsule is secreted anteriorly by epithelial cells and posteriorly by lens fibers that have their nuclei in the bow region (arrows) and their posterior processes in contact with the posterior capsule (fibers not drawn). The monolayer of epithelial cells is firmly attached to the anterior capsule. Epithelial cells proliferate, differentiate, and migrate to the equatorial region, and subsequently to the nuclear bow, to form fiber cells. (**B–L**) Sections of lenses were exposed to anti-SPARC IgG, followed by secondary antibody conjugated to FITC. (**B**) wt (+/+) at E11.5; (**C**) wt at E11.5, without primary antibody; (**D**) wt at E13.5; (**E**) SPARC-null (−/−) at E13.5; (**F**) wt at E17.5; (**G**) SPARC-null at E17.5; (**H**) wt, postnatal day 5; (**I**) wt, day 13; (**J**) wt at 1 month; (**K**) wt at 4 months; (**L**) wt at 18 months. Note that the staining intensity is stable in the lens epithelium up to 18 months of age. (**M**) Immunoblot of mouse lens epithelial cell protein, reactive with anti-SPARC IgG. Animal ages are indicated. Lane 1 represents murine SPARC (produced by parietal yolk sac cells) as a positive control. Lenticular SPARC migrated at 43 kD; fragments of lower molecular weight were not seen. mo, month; yr, year. Bars: **B–G** = 170 μm; **H–L** = 40 μm.

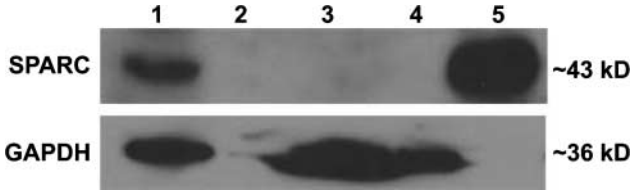

Figure 2 SPARC is not a component of the lens basement membrane. Western blots of lens proteins were incubated with anti-SPARC IgG. Lane 1, 2 wt lens capsules with epithelium; Lane 2, 2 wt capsules without lens epithelium; Lane 3, Decapsulized wt lens mass; Lane 4, 2 SPARC-null lens capsules with epithelium; Lane 5, SPARC control. The blot was reprobed with anti-GAPDH IgG. Note by the absence of GAPDH that the lens epithelial cells were completely removed from the capsules (Lane 2).

SPARC mRNA was present in both lens epithelial cells and in fiber cells (Figure 3G). Connexin 43 (Cx43) was expressed specifically in the undifferentiated lens epithelial cells and not in fiber cells (Donaldson et al. 1995). Therefore, Cx43 was used to detect epithelial cell contamination of the fiber cell isolates used for RT-PCR. The absence of Cx43 in the lens fiber cells indicates that the presence of SPARC mRNA in the fibers was not due to epithelial cell contamination.

Absence of SPARC in the Lens Is Associated with a Disrupted Capsule

The absence of SPARC in the lens capsule supports the contention that SPARC does not contribute to the

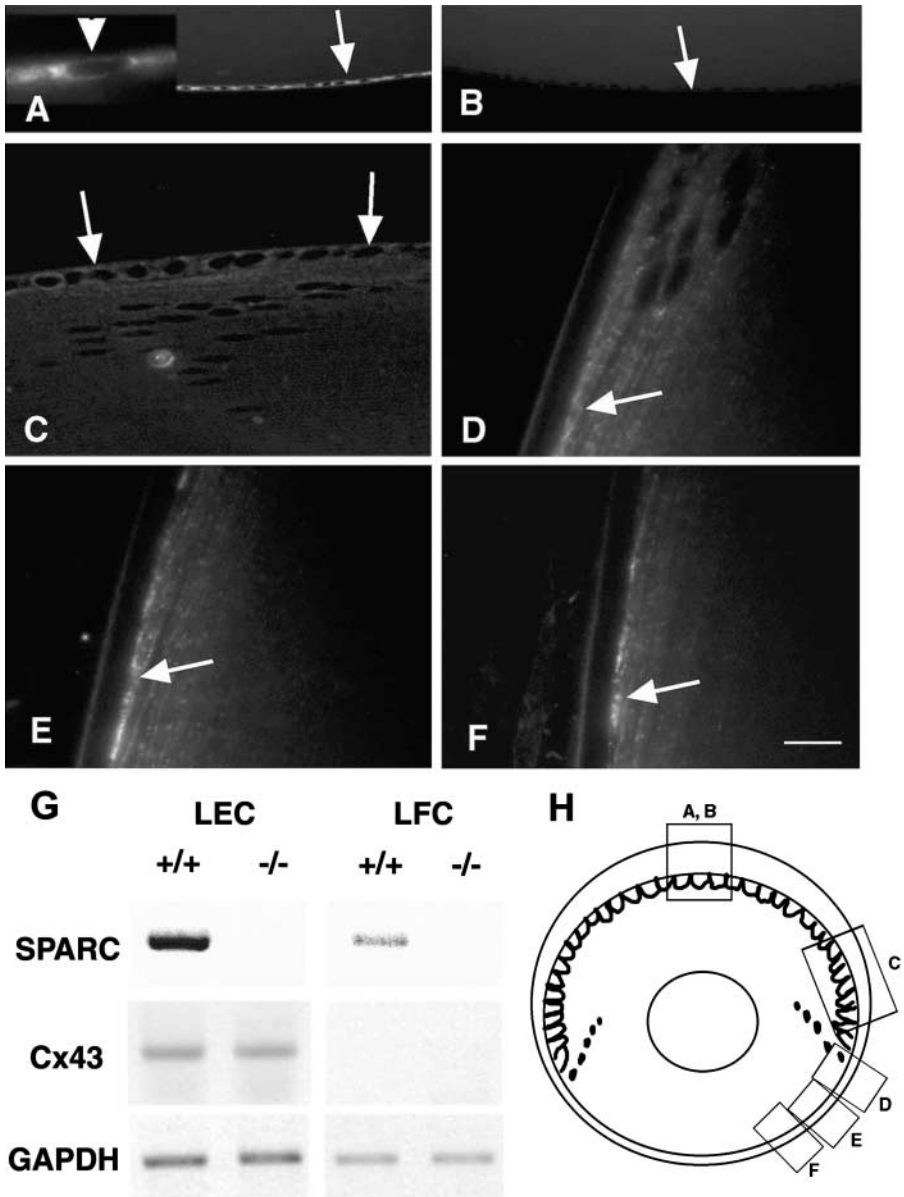

Figure 3 Localization of SPARC in lenses at 1 month of age. Immunohistochemical localization of SPARC protein in lenses of mice at 1 month of age. (**A**) Wild-type anterior lens section exposed to anti-SPARC IgG; cell nuclei were negative (arrowhead, inset). (**B**) SPARC-null lens section exposed to anti-SPARC IgG. (**C**) wt lens section immediately anterior to equator (see schematic, C). Labeling of SPARC in the epithelial cells in this region is minimal in comparison to the anterior epithelium shown in the schematic as A and B. (**D**) wt lens section posterior to equator; SPARC is located in the fiber cell posterior processes. (**E**) wt lens section posterior to **D**. (**F**) wt lens section posterior to **E**. (**G**) RT-PCR using primers for SPARC, Cx43, and GAPDH mRNAs in lens epithelial cells (LEC) and cortical fiber cells (LFC). Cx43, expressed specifically in the undifferentiated lens epithelial cells, was used to verify the lack of contamination of the fiber cells by epithelium. GAPDH transcript was present in all samples. (**H**) Schematic of a cross-section of the lens showing regions **A–F** (boxed), which correspond to the stained tissues (**A–F**). Bar: **A,B** = 40 μm; **C–F** and **inset** = 10 μm.

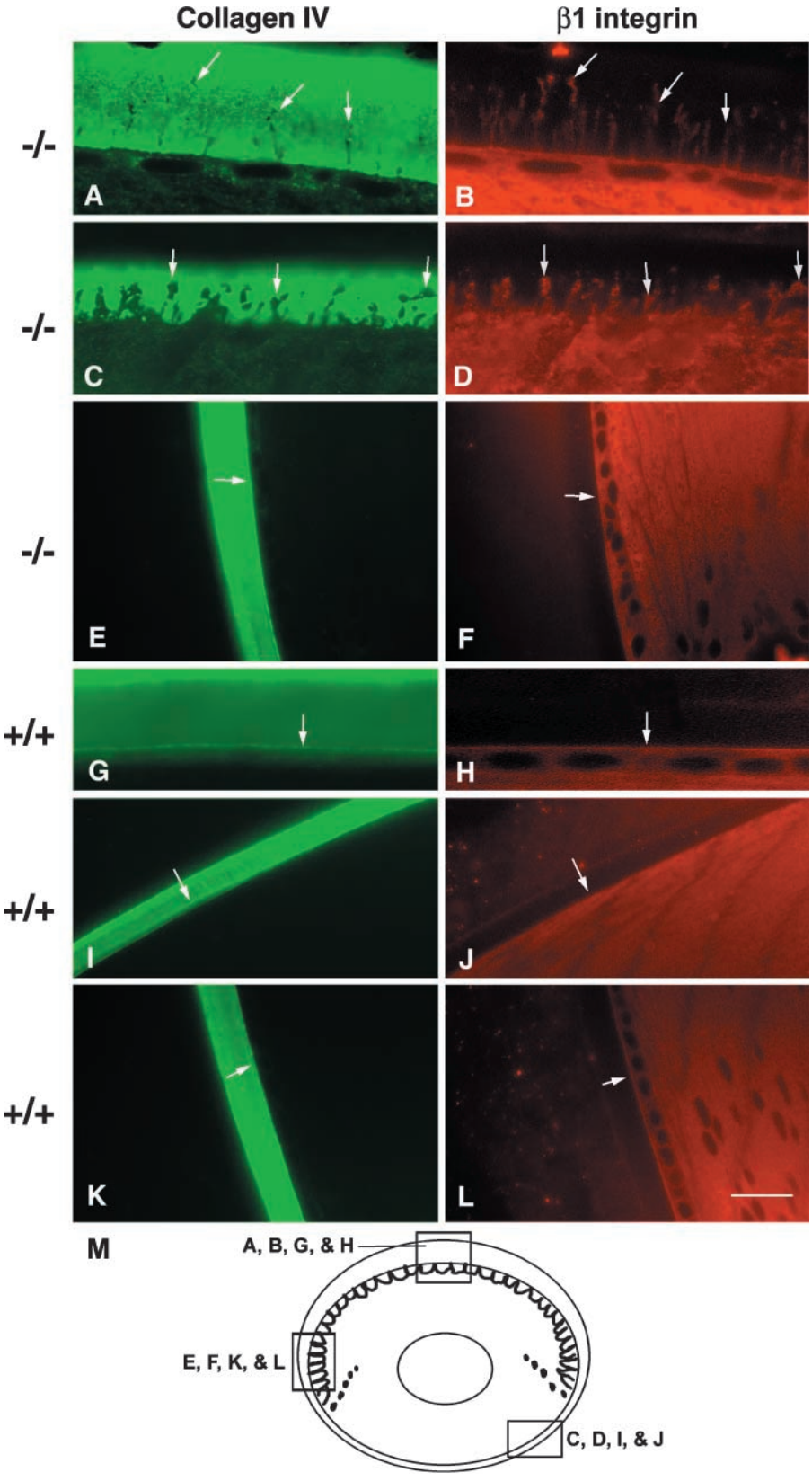

Figure 4 Protrusions of epithelial and fiber cells into the lens capsules of SPARC-null mice. Double-label immunofluorescence was performed on sections of SPARC-null (−/−) and wt (+/+) lenses from mice 2.5 months of age. Lens capsules were labeled with anti-collagen IV IgG (green, left panel), whereas the antibody against integrin-β_1 identified the cells and their processes (red, right panel). The clefts revealed by anti-collagen IV IgG were filled with cell processes labeled by anti-integrin-β_1 IgG (arrows; **A,B**, anterior region; **C,D**, posterior region). The region anterior to the equator showed no clefts in the capsule (**E**) and no protrusions from the cells (**F**). Sections of wt lenses (2.5 months) showed a smooth interface and intact capsule at the anterior (**G,H**) and posterior regions (arrows, **I,J**). The region anterior to the equator exhibited a similar interface and capsule as the corresponding area in the SPARC-null lens (**K,L**). (**M**) Schematic of a cross-section of the lens shows the regions **A–L** (boxed), which correspond to the stained tissues (**A–L**). Bar = 10 μm.

structure of this specialized ECM (Bornstein 1995; Bornstein and Sage 2002). Instead, SPARC could be an important protein in the regulation of the normal functions of both the lens epithelial cells and some of the peripheral fibers. Depletion of SPARC might be expected to alter the secretion, deposition, and assembly of some of the lens capsular ECM proteins at the basal end of lens fibers. Alternatively, the lack of SPARC might result in abnormal growth of lens cells, which could invade and degrade the basement membrane, a condition leading to disruption of the capsule (Yan et al. 2002).

In the SPARC-null mouse there is substantial damage of the lens capsule, with many protrusions of lens cells into the capsule (Yan et al. 2002; Norose et al. 2000). We asked whether the cellular localization of SPARC in normal lens was consistent with the damage seen in the mutant lens capsule. SPARC-null lens cells and capsule were double-stained with antibodies against collagen type IV and integrin-β_1 (Figure 4). In the SPARC-null tissue, the anterior capsule exhibited distinctive labeling with anti-collagen IV IgG (green), which surrounded many fine clefts that contained processes emanating from the basal surfaces of the epithelial cells (red) (Figures 4A and 4B, arrows). The damage observed in the posterior capsule is typically more severe than that in the anterior region. The enlarged clefts revealed by anti-collagen IV IgG contained larger fiber cell protrusions (arrows, Figures 4C and 4D). Interestingly, the region anterior to the equator that showed minimal production of SPARC in the epithelial cells by immunohistochemistry (see Figure 3C, wt lens) exhibited a continuous capsular structure and a smooth interface in the SPARC-null lens (Figures 4E, and 4F), an observation suggesting that the production of SPARC is correlated with the cellular invasion of the basement membrane and, possibly, with the aberrant capsular structure. The wt lens capsules were intact in both the anterior and posterior regions, with a smooth basal surface, and the lens cells did not exhibit protrusions into the capsules (Figures 4G–4L).

Discussion

SPARC is essential for the maintenance of lens transparency and lens capsular integrity. Significant labeling of SPARC was detected in the epithelial cells and newly differentiated fiber cells in the murine lens up to 2 years of age (Figure 1). It is known that the expression of SPARC is both temporally and spatially regulated in normal tissues (Lane and Sage 1994). Although abundant in embryonic tissues, SPARC is diminished in most postnatal or adult tissues (Sage et al. 1989). Therefore, the strong labeling of SPARC in lens epithelial cells in adult mice (Figure 1) appears to be an exception to data published for other tissues (Sage et al. 1989; Lane and Sage 1994). Lens epithelial cells grow, migrate, elongate, and differentiate into fiber cells throughout life (Kuzsak et al. 1999). The continuous presence of SPARC in adult lenses might be due to this unique growth pattern of the lens and indicates a role for SPARC in the normal activities of the lens cells.

Lens basement membrane is a highly specialized ECM present at the epithelial and fiber interface of the lens. It is now evident that individual components of the basement membrane regulate specific biological activities, such as cell growth, migration, adhesion, and differentiation, all of which contribute to the development of tissues (Timpl 1996; Aumailley and Gayraud 1998). All basement membranes contain type IV collagen, laminin, nidogen, and perlecan, which interact with each other and contribute to the supramolecular assembly of the basement membrane (Timpl and Dziadek 1986). Alterations in these components could lead to a deficient structure and to compromised biological responses of the cells that contact it. SPARC had been considered to be a minor component of certain basement membranes (Maillard et al. 1992). To our surprise, SPARC was not detected in the lens capsule, despite its abundance in an established basement membrane tumor, the EHS sarcoma (Dziadek et al. 1986; Mann et al. 1987). Therefore, the function of SPARC in the lens appears to involve regulation of epithelial cell function rather than acting as a stable component of the capsule.

The distribution of collagen type IV was altered in the SPARC-null lens (Yan et al. 2002). Whether or not this is a reflection of the damaged capsule and/or an altered expression of collagen caused by the deletion of SPARC needs to be investigated. Synthesis and/or assembly of collagen IV may be compromised in the SPARC-null lens, because the morphology of the lens cells, which normally produce capsular ECM proteins, is substantially altered (Norose et al. 2000; Yan et al. 2002).

The depletion of SPARC appears to be associated with cellular protrusions into the lens capsule by epithelial cells and fiber cells (Figure 4). Figure 5 is a drawing summarizing our results. (a) SPARC is present in the lens epithelial cells and newly differentiated fiber cells, but not in the lens capsule. (b) wt lens epithelial cells showing minimal reactivity with anti-SPARC IgG were located anterior to the lens equator (Figure 5, gray area; see also Figure 3C). The corresponding cells in the SPARC-null lens showed no cellular invasion into the basement membrane (Figures 4E, 4F, and 5). It has been reported that expression of SPARC is significantly reduced or absent in ovarian cancer (Yiu et al. 2001) and in some types of transformed cells that are highly invasive (Mettouchi et al. 1994; Vial and Castellazzi 2000). These reports, to-

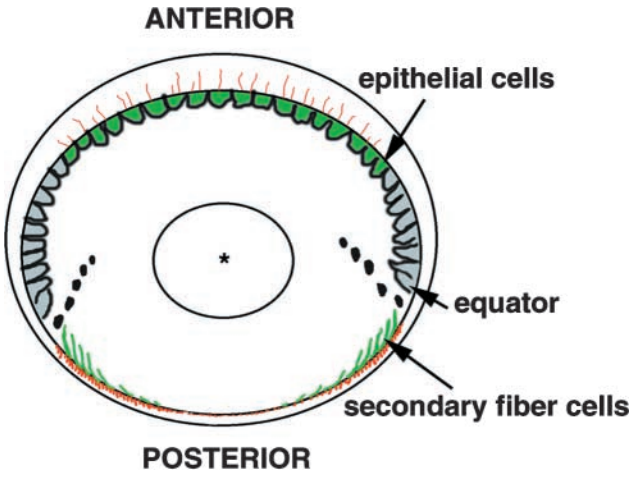

Figure 5 Schematic of a cross-section of the lens, showing the relationship between the distribution of SPARC in wt lens and the disruption of the capsule in SPARC-null lens. The green color represents the expression of SPARC in the epithelial cells (cell nuclei not drawn) and posterior processes of the newly differentiated fiber cells in wt lens. The gray color represents the diminished labeling of SPARC in the epithelial cells located in the region anterior to the equator in wt lens. Red represents cellular invasion from the lens cells into the capsule in SPARC-null lens. Note that the wt epithelial cells showing minimal reactivity with anti-SPARC IgG exhibited a smooth basal interface and no cellular extensions into the capsule, a characteristic reiterated in the corresponding epithelial cells in the SPARC-null lens. Lens components are not drawn to scale. Asterisk indicates primary lens fibers.

gether with our observations, suggest that SPARC diminishes invasion in some types of cells that normally contain abundant SPARC. The severe damage seen in the SPARC-null posterior capsule relative to the anterior capsule could be explained by the following possibilities. (a) Fiber cell protrusions are more substantial than epithelial cell protrusions. (b) The posterior capsule is thinner and therefore more labile. (c) SPARC is crucial for the process of fiber cell terminal differentiation; therefore, the absence of SPARC might lead to abnormal fiber formation.

Interestingly, the absence of SPARC causes no apparent pathology in the lens nucleus before mature cataract (Yan et al. 2002), although it is expressed in the embryonic primary fiber cells (Figure 1). It is possible that another SPARC family member, such as SC1 (McKinnon et al. 1996; Soderling et al. 1997), compensates for the loss of SPARC in the primary fiber cells, or that SPARC does not alter lens embryonic development. However, apparently, no other protein(s) can substitute for SPARC in lens epithelial cells, and in newly differentiated fiber cells, that would rescue the subsequently compromised lens capsule and disorganized secondary fibers (Yan et al. 2002).

The macromolecular structure of the basement membrane has become more complex as additional components are discovered and characterized. For example, the laminin family, collagen type XVIII, and nidogen-2 were recently described in several basement membranes (Erickson and Couchman 2000). The lens capsule has long been recognized as an important basement membrane that influences the biology of the lens. Although more studies are needed to identify both the components and their assembly in the lens capsule, the model of cataractogenesis in mice lacking SPARC will enable us to elucidate the role of SPARC in the formation and maintenance of the lens basement membrane.

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