The Matricellular Protein SPARC Decreases in the Lacrimal Gland At Adulthood and During Inflammation

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Purpose. Secreted protein acidic and rich in cysteine (SPARC) is a matricellular glycoprotein abundantly expressed in basement membranes and capsules surrounding a variety of organs and tissues. It mediates extracellular matrix organization and has been implicated in cell contraction. Here, we evaluated the expression of SPARC in the murine lacrimal gland at adulthood and during inflammation.

Methods. Lacrimal glands of young mice (4–6 weeks old) and adult mice (32–40 weeks old) were used for extraction of DNA, RNA, and protein. The presence of SPARC was assessed by quantitative PCR, ELISA, and immunofluorescence microscopy. 5-Methylcytosine and DNA methylation were evaluated using ELISA and bisulfite genomic sequencing, respectively. The effects of cytokines and inflammation in *Sparc* expression were evaluated in vitro and in the non-obese diabetic (NOD) mouse model of Sjögren's syndrome.

Results. The mRNA and protein levels of SPARC were downregulated in lacrimal glands of mature adult mice presenting age-related histological alterations such as increased deposition of lipofuscin and lipids. Epigenetic analyses indicated that glands in adult mice contain higher levels of global DNA methylation and show increased hypermethylation of specific CpG sites within the *Sparc* gene promoter. Analysis of smooth muscle actin (SMA)–green fluorescent protein (GFP) transgenic mice revealed that SPARC localizes primarily to myoepithelial cells within the gland. Treatment of myoepithelial cells with IL-1β or TNF-α and the development of inflammation in the NOD mice led to decreased transcription of *Sparc*.

Conclusions. SPARC is a novel matricellular glycoprotein expressed by myoepithelial cells in the lacrimal gland. Loss of SPARC during adulthood and chronic inflammation might have detrimental consequences on myoepithelial cell contraction and the secretion of tear fluid.

Keywords: adulthood, inflammation, lacrimal gland, myoepithelial cell, SPARC

The lacrimal gland is an exocrine gland responsible for secreting the aqueous portion of the tear film, a unique fluid covering the outer mucosal surfaces of the eye. Major functions of the tear film include lubrication of the ocular surface, providing protection against desiccation and environmental stress, and forming a refractive surface that allows light transmission into the retina.[1] The lacrimal gland is composed of multiple lobules separated from one another by loose connective tissue.[2] Each lobule contains many acini with pyramid-shaped epithelial cells and a central lumen. Similar to other glandular epithelia, these acini and intercalated ducts are surrounded by myoepithelial cells that border the basal lamina and separate the epithelial layer from the extracellular matrix.[3] These cells have structural features of both epithelial and smooth muscle cells and express α-smooth muscle actin (SMA) and cytokeratin 5. As a critical component of the lacrimal gland, myoepithelial cells synthesize components of the extracellular matrix and have contractile ability. The latter function is controlled by hormonal and neural mechanisms and is crucial to propel fluid secretions out of the lumen into the ocular surface.

Aging is tightly linked to the dysregulation of the immune system and the production of a constant low level of inflammation throughout the body. In the aging lacrimal gland, immunological alterations, in addition to genetic and hormonal factors, contribute to the impairment of secretory function.[4] Experiments in animal models of aging have shown that the aging lacrimal gland exhibits an increased number of lymphocytic infiltrates with a reduction in neural and agonist-stimulated tear secretion, and it shows morphological alterations such as the deposition of lipofuscin-like material.[5,6] These findings have contributed to the principle that aging of the lacrimal gland is a major risk factor for developing aqueous-deficient dry eye disease.[7] One frequent

finding in the aging population is the increased incidence of autoimmunity, a classic example being Sjögren's syndrome. This systemic disease is characterized by the immune-related dysfunction of both lacrimal and salivary glands, which leads to chronic dry eye and dry mouth, respectively. Recent experiments performed in animal models of Sjögren's syndrome have revealed that their lacrimal glands display an impairment in myoepithelial cell-driven acini contraction.[8] Consequently, new efforts have focused on identifying the mechanisms driving the contraction of myoepithelial cells in health and disease.

Secreted protein acidic and rich in cysteine (SPARC), also known as osteonectin or basement-membrane protein 40, is a calcium- and collagen-binding protein belonging to the family of matricellular proteins.[9] These proteins do not serve structural roles but are secreted into the microenvironment where they modulate the interaction of cells with the extracellular matrix. SPARC has a molecular weight of 34 kDa and consists of three structural domains implicated in the regulation of cellular adhesion and extracellular matrix organization.[10] This protein has been found in basement membranes and in capsules that surround a number of organs and tissues.[11] In the eye, SPARC has been detected in the epithelia and capsule of the lens, and its deficiency has been associated with lenticular opacity at an early age that progresses gradually into mature cataracts.[12,13] Strong expression of SPARC protein has also been observed in myoepithelial cells of breast cancer patients[14] and has been implicated in the contractile function of fibroblasts and cardiomyocytes.[15,16] Here, we show that SPARC is downregulated in lacrimal glands of adult mice most likely due to epigenetic changes related to the hypermethylation of CpG sites within the *Sparc* gene promoter. We identified, using transgenic mice, myoepithelial cells as the primary cellular site responsible for the synthesis of SPARC in the lacrimal gland. We further demonstrated that treatment of myoepithelial cells with proinflammatory cytokines and development of inflammation in an animal model of Sjögren's syndrome lead to decreased transcription of *Sparc*. These results might have important implications for the regulation of myoepithelial cell contraction in the lacrimal gland at adulthood and during inflammatory conditions.

Materials and Methods

Animals

All animal-based procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The experimental protocols were approved by the Animal Care Committee of the Schepens Eye Research Institute of Mass. Eye and Ear and the Tufts Medical Center Animal Care and Use Committee. Female and male C57BL/6J, male BALB/c, and male NOR/LtJ mice were obtained from Charles River Laboratories (Wilmington, MA, USA) and from The Jackson Laboratory (Bar Harbor, ME, USA). Female and male smooth muscle actin (SMA)–green fluorescent protein (GFP) reporter mice (C57BL6/SMA^{CreErt2} strain) described by Yokota et al.[17] were a kind gift of Ivo Kalajzic at UConn Health (Farmington, CT, USA). Two groups of C57BL/6J mice, young (4–6 weeks old) and mature adult (32–40 weeks old; no retired breeders) were used. *Sparc* expression was also measured in lacrimal glands of 17- to 18-week-old C57BL/6J mice. The exorbital lacrimal glands were removed and processed for histological examination and extraction of DNA, RNA, and protein as described below.

Cell Culture

Exorbital lacrimal glands from 4- to 6-week-old SMA–GFP mice were removed and minced into lobules for collagenase digestion as previously described.[18] The resulting cells were centrifuged at 100*g* for 5 minutes; resuspended in 10 mL of RPMI 1640 Complete Medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 100 µg/mL penicillin–streptomycin; and cultured in 100-mm culture dishes (VWR International, Radnor, PA, USA) at 37°C. GFP$^+$ myoepithelial cells were placed in eight-well cell chambers and fixed with ice-cold methanol for 10 minutes for immunostaining experiments. Alternatively, confluent GFP$^+$ myoepithelial cells were placed in six-well plates and treated with 10 ng/mL IL-1β or TNF-α for 7 days. The media were changed every other day with the addition of fresh cytokines.

Histology and Immunofluorescence

Oil Red O staining was performed on cryosections (6 µm) of the lacrimal gland. Sections were fixed in 10% formalin for 10 minutes at room temperature. After being washed in 60% isopropanol, the sections were incubated with freshly prepared Oil Red O working solution (Oil Red O Staining Kit; BioVision, Milpitas, CA, USA) following the manufacturer's protocol. For carbol fuchsin staining of lipofuscin, the cryosections were stained following the Remel TB Kinyoun Carbolfuchsin protocol (Thermo Fisher Scientific, Waltham, MA, USA).[5] Sections were fixed in absolute methanol for 10 minutes at room temperature before being stained with Kinyoun's carbol fuchsin solution for 5 minutes. The sections were rinsed in 50% ethanol for a few seconds, washed in distilled water, and decolorized in 1% sulfuric acid. For hematoxylin and eosin (H&E) staining, the lacrimal glands were fixed overnight with 4% formaldehyde in phosphate buffered saline (PBS), dehydrated, and embedded in paraffin. Sections (6 µm) were deparaffinized, rehydrated using graded alcohols, and processed for H&E staining following standard protocols.

Immunofluorescence was performed on cryosections of lacrimal gland tissue and methanol-fixed myoepithelial cell cultures from SMA–GFP mice. The slides were placed in a 55°C oven to dry for 10 minutes. Samples were blocked with 10% normal donkey serum (Jackson ImmunoResearch, West Grove, PA, USA) for 1 hour at room temperature and then incubated with an anti-SPARC antibody (1:100, ab203284; Abcam, Cambridge, UK) diluted in 1% bovine serum albumin in PBS at 4°C overnight. After the slides were washed with PBS, they were incubated with an anti-IgG Alexa Fluor 555 antibody (ab150062; Abcam) for 1 hour at room temperature. Coverslips were mounted onto the slides using Invitrogen SlowFade Diamond Antifade Mountant with DAPI (Thermo Fisher Scientific). Tissue sections were analyzed using an A1R Confocal Microscope (Nikon, Tokyo, Japan) and cell cultures using an Eclipse E600 fluorescence microscope (Nikon).

RNA Isolation and Quantitative Real-Time PCR

Lacrimal gland tissue was homogenized using a manual pestle, and total RNA was isolated using an extraction

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reagent (Invitrogen TRIzol; Thermo Fisher Scientific) according to the manufacturer's protocol. Total RNA from cultured lacrimal gland myoepithelial cells was isolated using the RNeasy Plus Micro Kit (QIAGEN, Hilden, Germany). Total RNA was transcribed using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). Quantitative real-time PCR (qPCR) was performed using the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad). Primer sequences for *Sparc* (Unique Assay ID qMmuCID0023536) and *Gapdh* (Unique Assay ID qMmuCED0027497) mRNA were obtained from Bio-Rad. Gene expression was measured in a Stratagene Mx3000 Multiplex Quantitative PCR system (Agilent, Santa Clara, CA, USA) with the following parameters: 2 minutes at 95°C, followed by 40 cycles of 5 seconds at 95°C and 30 seconds at 60°C. Fold changes were calculated using the comparative C_T ($\Delta\Delta C_T$) method by normalizing to *Gapdh*.

SPARC ELISA

Lacrimal glands were placed in radioimmunoprecipitation assay buffer (10-mM Tris-HCl, pH 7.4; 150-mM NaCl; 1-mM EDTA; 1% Triton X-100; 0.1% sodium deoxycholate; 10-mM dithiothreitol; and 0.1% SDS) supplemented with a protease inhibitor cocktail (Sigma-Aldrich). The tissue was homogenized with a manual pestle for approximately 1 minute at room temperature. After centrifugation at 16,000*g* for 5 minutes, the total protein concentration was determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). The SPARC ELISA (ABclonal Technology, Woburn, MA) was carried out according to the manufacturer's protocol.

Genomic DNA Isolation

The genomic DNA (gDNA) was isolated using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). The tissue was placed in 300 µL of nuclei lysis solution and homogenized using a manual pestle. Samples were incubated with 3 µL of RNase solution at 37°C for 20 minutes, cooled down to room temperature, and then treated with 200 µL of protein precipitation solution. The samples were incubated on ice for 5 minutes before centrifugation at 21,000*g* for another 5 minutes. The supernatant was placed in a fresh tube containing 600 µL of isopropanol at room temperature, gently mixed by inversion, and subjected to another centrifugation step. The supernatant was removed and mixed with 600 µL of 70% ethanol. After gentle mixing and a centrifugation step, the ethanol was aspirated and the pellet air dried. The gDNA was rehydrated in 30 µL of DNase-free water and stored at 4°C. The total concentration of gDNA was measured using a NanoDrop 1000 (Thermo Fisher Scientific).

Methylation Assays

The levels of 5-methylcytosine (5-mC) in mouse tissues were determined using the MethylFlash Global DNA Methylation ELISA Easy Kit (EpiGentek, Farmingdale, NY, USA) according to the manufacturer's protocol. Methylation analyses of four CpG sites residing within the *Sparc* gene promoter region[19] were carried out using the EpiTect Bisulfite Kit (QIAGEN). Unmethylated cytosine was converted to uracil by bisulfite treatment of 1 µg of gDNA. The bisulfite-modified DNA was amplified with primers for the *Sparc* promoter (forward, 5′-GGGTTGGAATAGTTGTTGGAA-3′; reverse, 5′-GAGTGAATTTGTTTGAGTATTTTT-3′) using ZymoTaq DNA Polymerase (Zymo Research, Freiburg, Germany). The amplified DNA was purified with the Wizard SV Gel and PCR Clean-Up System (Promega). The extracted product was ligated (1:10 molecular weight ratio to the vector) into pSTBlue-1 using the pSTBlue-1 AccepTor Vector Kit (Novagen, Madison, WI, USA). After ligation, 1 µL of the product was used to transform NovaBlue Singles Competent Cells (Novagen). Plasmid DNA was isolated from positive colonies using the Monarch Plasmid Miniprep Kit (New England BioLabs, Ipswich, MA, USA), and 1 µg of the plasmid was sent to Eurofins Clinical Enterprise (Framingham, MA, USA) for sequencing. The results were analyzed using ApE Software (Madera, CA, USA) and BiQ Analyzer for visualization of the DNA methylation data.

Statistical Analyses

Statistical analyses were performed using Prism 9 for Macintosh (GraphPad Software, San Diego, CA, USA). Significance was determined using the Mann–Whitney test or nonparametric Kruskal–Wallis test with Dunn's test for multiple comparisons. $P < 0.05$ was considered significant.

Results

SPARC Is Downregulated in the Lacrimal Gland At Adulthood

The main lacrimal glands in adult mice are located subcutaneously anterior to, and slightly below, the ears (Fig. 1A). These exorbital glands move the tear fluid into the ocular surface through a single excretory duct. It has been known for many years that aging is involved in a number of structural and functional alterations of the main lacrimal gland in both humans and experimental animals.[4] In mice, these include the appearance of lipofuscin-like inclusions, a post-mitotic pigment deposition traditionally associated with aging, at 32 weeks and the development of chronic inflammation and functional alterations, including decreased acetylcholine release and impaired protein secretion.[5] In accordance with these data, we found extensive staining of carbol fuchsin in lacrimal glands of 32- to 40-week-old adult mice compared to young 4- to 6-week-old mice (Fig. 1B). Further histological examination by Oil Red O staining, another indicator associated with the progressive decline of the lacrimal gland,[20–22] demonstrated the accumulation of lipid droplets in the aging tissue. It should be noted here that we use the term aging in the context of biological changes that occur with time across the lifespan. Using lacrimal glands from these two groups of young and adult mice, we measured the amount of SPARC at the mRNA and protein levels. As shown in Figure 2A, we detected a significant downregulation in the number of *Sparc* transcripts in adult mice compared to young mice. Interestingly, we found decreased expression of *Sparc* in the lacrimal glands of 17- to 18-week-old mice, suggesting that this alteration occurs as the mice transition from a young age to an adult stage. This reduction in gene expression was concomitant with a reduced amount of SPARC protein in tissue extracts from animals in the mature adult group (Fig. 2B).

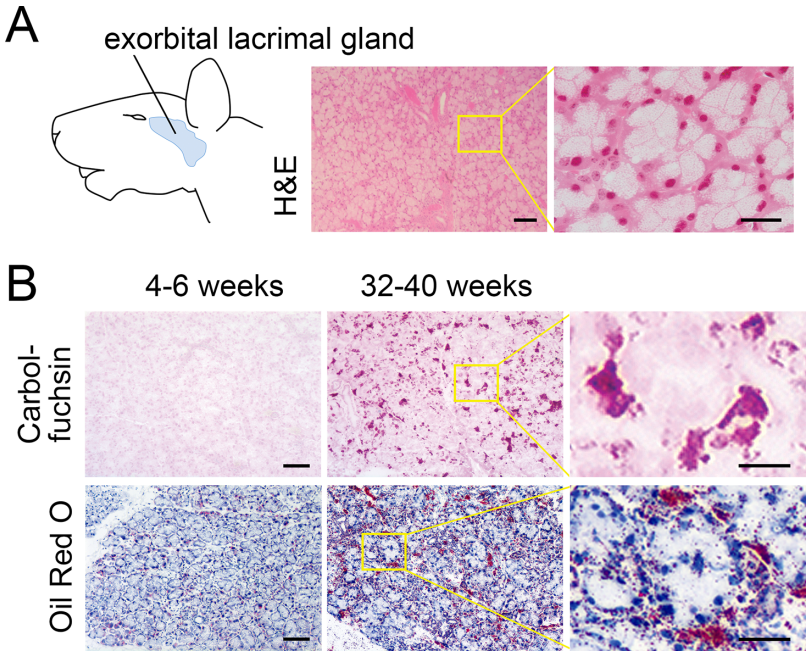

Figure 1. Age-related histological alterations in lacrimal glands of adult mice. (**A**) H&E staining of the exorbital lacrimal gland in C57BL/6J mice. The magnified area shows acini formed by epithelial cells and their nuclei. (**B**) Carbol fuchsin and Oil Red O staining of lacrimal gland cryosections from young (4–6 weeks old) and adult (32–40 weeks old) C57BL/6J mice. *Scale bars*: 100 µm (zoomed-in portion, 25 µm).

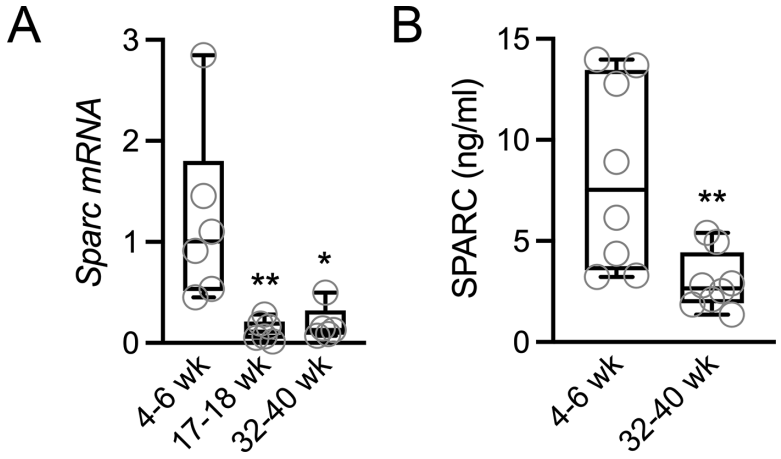

Figure 2. SPARC is downregulated in the adult lacrimal gland. (**A**) Total RNA was isolated from exorbital lacrimal glands from C57BL/6J mice at different ages (4–6 weeks old, 17–18 weeks old, and 32–40 weeks old; $n = 5$ or 6 mice per group). The relative levels of *Sparc* expression were determined using qPCR. (**B**) Total protein extracts were prepared from homogenized exorbital lacrimal glands from young (4–6 weeks old) and adult (32–40 weeks old) C57BL/6J mice and analyzed using ELISA ($n = 8$ mice/group). The box-and-whisker plots show the 25th and 75th percentiles (*boxes*), the median, and the minimum and maximum data values (*whiskers*). Significance was determined using the non-parametric Kruskal–Wallis test with Dunn's test for multiple comparisons (**A**) or the Mann–Whitney test (**B**). $^{*}P < 0.05$; $^{**}P < 0.01$.

Sparc Promoter Methylation Increases in the Adult Lacrimal Gland

DNA methylation is a common epigenetic modification involving primarily the transfer of a methyl group onto the C5 position of the cytosine nucleotide to generate 5-mC (Fig. 3A). This modification accounts for approximately 1% of nucleic acids in the human genome and occurs on cytosines that precede a guanine nucleotide or CpG sites, often at gene promoters.[23] Age-associated methylation changes to the genomic DNA are well documented and thought to promote diseases associated with aging by dictating the level of gene expression.[24] Because increased methylation of the *Sparc* promoter has been associated with decreased SPARC expression,[19,25] we evaluated the methylation status of the *Sparc* gene promoter in the lacrimal gland.

First, we determined the overall methylation levels by measuring 5-mC from extracted gDNA in the tissue. As

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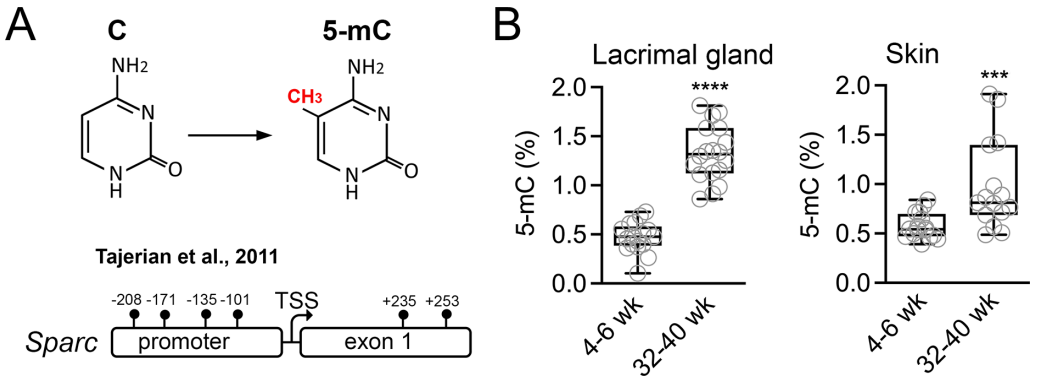

Figure 3. The levels of 5-mC increase in the adult lacrimal gland. (**A**) Diagrams depicting the methylation (*red*) of the DNA base cytosine (**C**) into 5-mC (*above*) and the *Sparc* promoter region with specific CpG sites (*below*). TSS, transcription start site. (Adapted from Tajerian et al.[19]) (**B**) The gDNA was isolated from exorbital lacrimal glands ($n = 18$ mice/group) and skin ($n = 16$ mice/group) from young (4–6 weeks old) and adult (32–40 weeks old) C57BL/6J mice. The levels of 5-mC were determined using ELISA. The box-and-whisker plots show the 25th and 75th percentiles (*boxes*), the median, and the minimum and maximum data values (*whiskers*). Significance was determined using the Mann–Whitney test. ***$P < 0.001$; ****$P < 0.0001$.

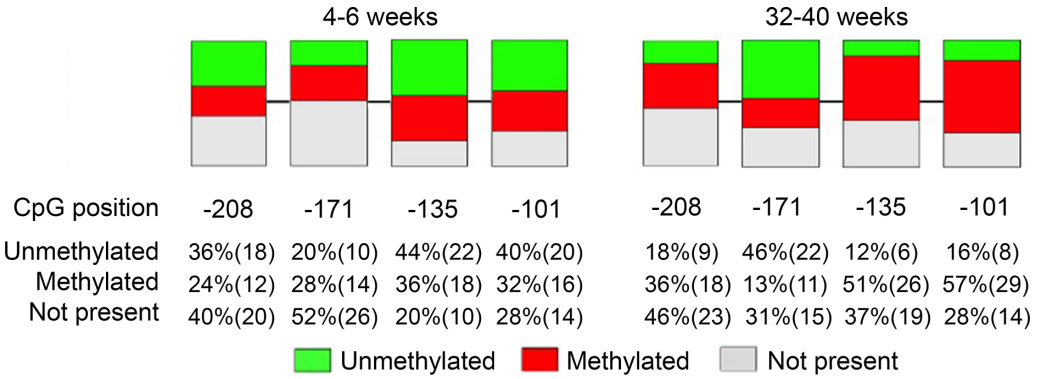

Figure 4. Methylation state of CpG sites in the *Sparc* gene promoter region. The gDNA was isolated from exorbital lacrimal glands ($n = 6$ mice/group) from young (4–6 weeks old) and adult (32–40 weeks old) C57BL/6J mice. The methylation of four specific CpG sites in the *Sparc* gene promoter region (−208, −171, −135, and −101) was evaluated by bisulfite genomic sequencing after PCR amplification and cloning into a vector. The graph shows the percentage of methylated and unmethylated sites in each group of mice, as well as the total number of sequences analyzed (in parentheses).

shown in Figure 3B, we observed a significant increase in methylation levels in lacrimal glands of 32- to 40-week-old adult mice compared to those in the young 4- to 6-week-old group. This increase toward DNA hypermethylation, although less pronounced, was also observed in skin samples of older animals, as previously reported.[26,27] Second, we performed bisulfite genomic sequencing to determine the methylation status in the *Sparc* gene promoter by using specific primers. This method constitutes a quantitative and efficient approach to identifying 5-mC at a single base-pair resolution.[28] We focused on the methylation state of four established CpG sites residing in the SPARC gene promoter region, particularly those in positions −208, −171, −135, and −101.[19] As shown in Figure 4, three CpG sites in positions −208, −135, and −101 showed increased levels of methylation in the adult lacrimal gland, whereas position −171 was hypomethylated. Analysis of the sequenced clones revealed a 12% increase in the methylation of the −208 site in older tissue, along with a 15% increase in the −135 site and a 25% increase in the −101 site. The methylation status of the −171 site was reduced by 15% in the adult gland.

SPARC Localizes to Myoepithelial Cells and Decreases During Inflammation

In subsequent experiments, we made use of SMA–GFP reporter mice to gain insight into the localization of SPARC in the murine lacrimal gland. Myoepithelial cells in this transgenic strain express GFP under the control of the SMA promoter and can therefore be easily identified.[18] As shown in Figure 5A, SPARC colocalized with GFP-expressing myoepithelial cells in the lacrimal gland and was found with a punctate distribution within the extracellular space, suggesting that SPARC is being secreted by these cells. The expression of SPARC by myoepithelial cells was further confirmed in colocalization experiments using cultured cells isolated from lacrimal glands of SMA–GFP mice (Fig. 5B).

We have recently reported that proinflammatory cytokines and chronic inflammation negatively affect the contractile function of lacrimal gland myoepithelial cells.[8,29] Consequently, we evaluated both in vitro and in vivo how inflammation affects the expression of *Sparc*. For these experiments, the levels of *Sparc* transcription

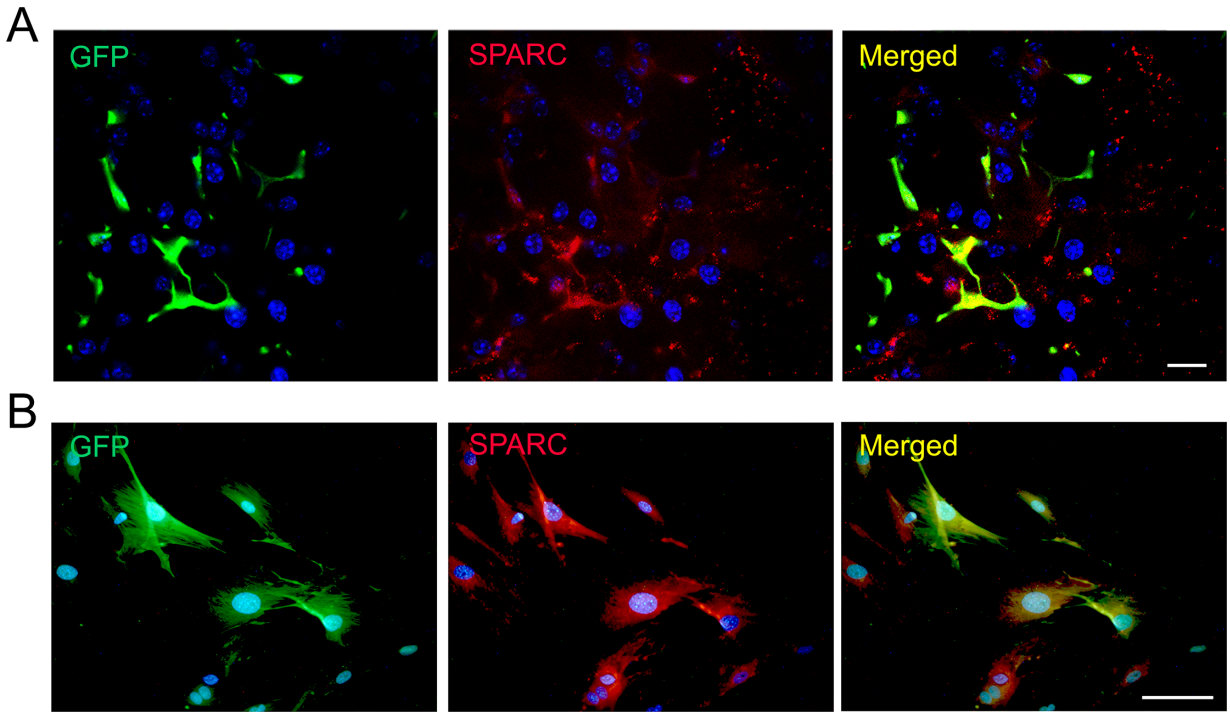

Figure 5. SPARC localizes to lacrimal gland myoepithelial cells. (**A**) Cryosections of lacrimal gland tissue from 4-week-old SMA–GFP mice were incubated with an anti-SPARC antibody. Merged images show colocalization of SPARC (*red*) with GFP-expressing myoepithelial cells (*green*). A fine punctate staining of SPARC can be observed within the extracellular space. (**B**) Immunostaining of SPARC (*red*) in cultured myoepithelial cells (*green*) isolated from lacrimal glands of 4- to 6-week-old SMA–GFP mice. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI; *blue*). *Scale bars*: 50 µm.

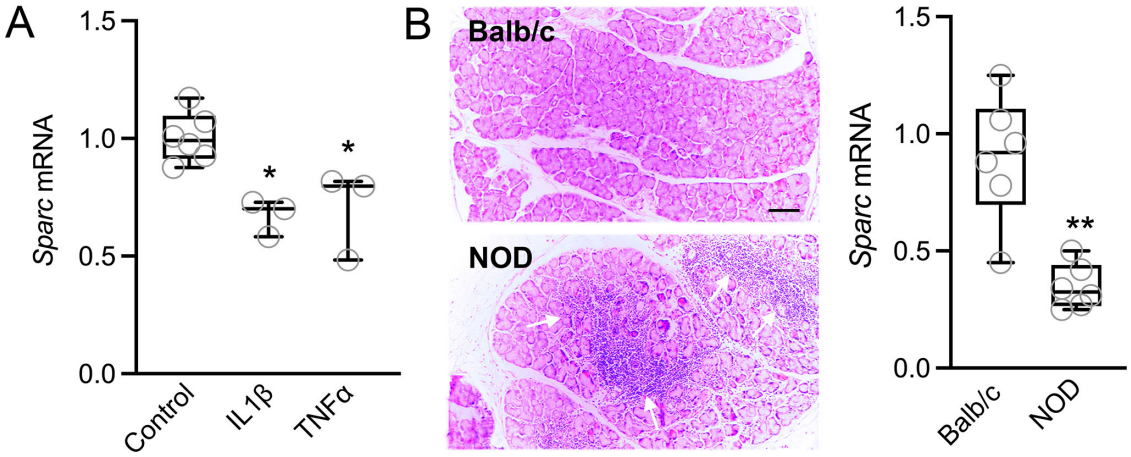

Figure 6. *Sparc* transcription decreases during inflammatory conditions. (**A**) Total RNA was obtained from myoepithelial cells isolated from lacrimal glands of SMA–GFP mice. The cells were cultured with IL-1β, TNF-α, or vehicle control. The relative levels of *Sparc* expression were determined using qPCR (n = 3–6 independent experiments). (**B**) H&E staining of the exorbital lacrimal glands of control 16-week-old BALB/c mice and 16-week-old NOD mice. Lacrimal glands from BALB/c mice show normal morphology with lobules devoid of immune cells. In contrast, lacrimal glands from NOD mice are heavily infiltrated by immune cells that formed large foci within the lobules (*arrows*). *Scale bar*: 100 µm. Total RNA isolated from the lacrimal glands of these mice was used to determine the relative levels of *Sparc* expression using qPCR (n = 6 mice/group). The box-and-whisker plots show the 25th and 75th percentiles (*boxes*), the median, and the minimum and maximum data values (*whiskers*). Significance was determined using the non-parametric Kruskal–Wallis test with Dunn's test for multiple comparisons (**A**) or the Mann–Whitney test (**B**). *$P < 0.05$; **$P < 0.01$.

were first evaluated in GFP$^+$ myoepithelial cells isolated for lacrimal glands of SMA–GFP mice. These cells were incubated with IL-1β or TNF-α for 7 days. As shown in Figure 6A, treatment with proinflammatory cytokines led to a significant downregulation in the transcription of *Sparc*. Next, we measured the level of *Sparc* expression in the non-obese diabetic (NOD) mouse model of Sjögren's syndrome. These mice display severe inflammation with prominent areas in the exorbital lacrimal gland covered by lymphocytic foci (Fig. 6B).[30] Relative quantification of *Sparc* transcription in

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lacrimal glands isolated from 16-week-old mice revealed a significant decrease as compared to age-matched control animals.

Discussion

The lacrimal gland has remained a major focus of research because of the implications of its dysfunction to the development of aqueous-deficient dry eye disease. Here, we aimed to evaluate whether the expression of SPARC, a matricellular glycoprotein involved in extracellular matrix organization and cell contraction, is altered in the lacrimal gland at adulthood and during inflammation. Our results demonstrate transcriptional and epigenetic variation of *Sparc* in the adult mice and identify lacrimal gland myoepithelial cells as the source of SPARC biosynthesis. We also present evidence indicating that IL-1β, TNF-α, and the development of inflammation lead to decreased transcription of *Sparc*. These results have potential implications on the normal functioning of the lacrimal gland and uncover SPARC as a specific molecule altered by biological aging and inflammation.

Myoepithelial cells surround ducts and acini of glandular organs and constitute a biological boundary separating proliferating epithelial cells from basement membranes and underlying stroma. Their contraction is controlled by activation of the oxytocin receptor by the oxytocin neurotransmitter and is crucial for fluid ejection.[18] These cells express several contractile proteins such as SMA and calponin and morphologically resemble smooth muscle cells.[3] We hypothesize that decreased SPARC expression in our experiments has implications on the contractile function of lacrimal gland myoepithelial cells and, consequently, the secretion of tear fluid into the ocular surface. This hypothesis is based on a number of observations. First, SPARC is expressed in basement membranes and in capsules surrounding a variety of organs and tissues,[11] a location that would allow direct control of the secretory function in glandular epithelia. Second, the expression of SPARC has been directly associated with the contractile function of cells. For example, extracellular SPARC increases cardiomyocyte contraction in health and disease conditions through interaction with the β1 integrin/integrin-linked kinase complex on the cardiomyocyte membrane.[16] SPARC also appears to be important to the contractile function of the tendon. Loss of SPARC results in defective tendon collagen fibrillogenesis, and tendons from *Sparc* null mice are less able to withstand force in comparison with control mice.[31] A close examination of the *Sparc* null mice in future studies should contribute to better elucidating the role of this protein in lacrimal gland function and the development of dry eye disease. To the best of our knowledge, no ocular surface phenotype has been reported in the *Sparc* null mice, despite several studies reporting ocular alterations following gene abrogation.[32] However, the presence of any potential sign of dry eye disease in these mice might not be obvious and will likely require precise controlled environmental conditions and the use of specific tests to evaluate ocular surface damage.

There is strong evidence linking alterations in DNA methylation with aging and age-related diseases.[33] These alterations are known to induce changes in gene expression by recruiting proteins involved in gene repression or inhibiting the binding of transcription factors to DNA promoter sequences near transcription start sites.[23] Changes in the methylation status of the *Sparc* gene promoter have been reported primarily in tumors. They mostly involve hypermethylation and have been linked to the decreased expression of protein, both in vitro and in vivo.[25,34–36] In fewer instances the *Sparc* gene promoter has been found to be hypomethylated, leading to overexpression of the protein. This has been the case in breast cancer, where high expression of SPARC induced by promoter hypomethylation has been shown to promote cell migration and invasion.[37] Of interest to our study are previous findings showing hypermethylation of the *Sparc* gene promoter and decreased synthesis of SPARC in aging mice, which has been linked to degeneration of the intervertebral discs and chronic low back pain.[19] Our results showing increased overall methylation levels in the adult lacrimal gland are in line with these findings and identified *Sparc* gene hypermethylation at three out of four CpG sites analyzed. It is plausible that these epigenetic changes are directly responsible for the reduced expression of SPARC observed in the lacrimal gland, independently of any alteration in myoepithelial cell numbers.

One of the major transformations that occur with biological aging is the dysregulation of the immune system and the establishment of a proinflammatory environment. Cytokines and chemokines are important proinflammatory mediators responsible for the development of chronic inflammation and the immunosenescence process.[38] IL-1β is upregulated in acinar cells prepared from lacrimal glands infiltrated with lymphocytes, such as those from murine models of Sjögren's syndrome, and both IL-1β and TNF-α have been shown to inhibit neurally mediated lacrimal gland secretion.[39] More recent research indicates that proinflammatory cytokines affect the contractile ability of myoepithelial cells in the lacrimal gland and may account for the reduced tear secretion observed in Sjögren's syndrome.[29] Our data suggest that proinflammatory cytokines also contribute to the impairment of tear fluid secretion by reducing the expression of *Sparc* in lacrimal gland myoepithelial cells. In line with these findings, we also find that the number of *Sparc* transcripts is downregulated in the NOD mouse model of Sjögren's syndrome. Understanding how SPARC can be therapeutically modulated could have important implications for the secretory function of the lacrimal gland during pathological conditions.

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