SPARC is expressed in scars of the Tenon’s capsule and mediates scarring properties of human Tenon’s fibroblasts in vitro

Rudolf Fuchshofer,1 Ulrike B. Kottler,2 Anne V. Ohlmann,3 Ursula Schlötzer-Schrehardt,2 Anselm Jünemann,2 Friedrich E. Kruse,2 Andreas Ohlmann1,2

(The first two authors contributed equally to this work)

1Institute of Human Anatomy and Embryology, University of Regensburg, Regensburg, Germany; 2Department of Ophthalmology, University Erlangen-Nürnberg, Erlangen, Germany; 3Department of Ophthalmology, Ludwig-Maximilians-University, Munich, Germany

Purpose: To investigate the expression of the matricellular protein SPARC (secreted acidic cysteine-rich glycoprotein) in scarred human Tenon’s capsule and in cultured human Tenon’s fibroblasts (HTF), and to analyze the influence of SPARC on cell proliferation and collagen matrix contraction in vitro.

Methods: Human Tenon's capsule scars obtained from surgical revisions after filtration surgery were analyzed for SPARC expression by immunohistochemistry. In cultured HTF cells, SPARC expression was assessed by northern and western blot analyses after incubation with transforming growth factor (TGF)-β1 and TGF-β2. Cell proliferation was determined by bromodeoxyuridine (BrdU)–labeling and HTF cells-mediated collagen matrix contraction by morphometric measurements of three-dimensional collagen lattices after treatment with SPARC and/or TGF-β1.

Results: In scarred human Tenon’s capsule specimens, an increased expression of SPARC was mainly localized to the extracellular matrix and to blood vessel walls as compared to healthy control Tenon’s capsule. In cultured HTF cells, treatment with TGF-β1 more than TGF-β2 induced the expression of SPARC both on the mRNA and protein level. Incubation of HTF cells with SPARC resulted in an increase in collagen matrix contraction and cell proliferation. Moreover, a combined incubation of SPARC and TGF-β1 stimulated HTF cell proliferation significantly over the levels that were observed after single treatment.

Conclusions: Our data provide evidence that SPARC contributes to excessive wound healing and scar formation in human Tenon’s capsules after filtration surgery and may thus represent a novel target for anti-fibrotic strategies.
METHODS

Sample collection and cell culture: Sample collection was approved by the local ethic committee and was performed after informed consent of patients, following the tenets of the Declaration of Helsinki. Biopsies of healthy human Tenon’s capsule and Tenon’s capsule scars were obtained from patients with ocular or systemic disease, such as inflammatory diseases or diabetes mellitus were excluded from the study.

Cultures of human Tenon’s fibroblast were established as described previously [29]. Briefly, Tenon’s capsule biopsies from five different healthy-controls were dissected, placed in 50 ml tissue-culture flasks in Dulbecco's modified Eagle's medium (DMEM/Ham's F12; Invitrogen, Karlsruhe, Germany) containing 15% (v/v) fetal calf serum and antibiotic-antimycotic solution (10,000 U/ml penicillin, 10,000 μg/ml streptomycin and 25 μg/ml amphotericin B; all from Invitrogen), incubated at 37 °C in a humidified 95% air/5% CO₂ atmosphere and fed every 3 days. Prior to experiments, HTF were starved 24 h in serum free culture medium. For all assays, serum free cell culture medium and 2nd-4th passage cells were used.

Proliferation assay: Cell proliferation assays of human Tenon’s fibroblast were performed by using bromodeoxyuridine (BrdU)–labeling of dividing cells according to manufacturer’s instructions (Roche, Mannheim, Germany). In brief, cells were seeded into 96-well plates at an initial density of 2×10³ cells/well and allowed to attach for 24 h. After another 24 h in serum free medium, the cells were treated with various concentrations of human recombinant SPARC and/or activated TGF-β1 (200 pg/ml; both R&D Systems, Wiesbaden, Germany). After 3 days, cells were fixed and incorporated BrdU was detected by enzyme-linked immunosorbent assay (ELISA) using an ELISA plate reader (Tecan, Crailsheim, Germany) at 450 nm.

Collagen contraction assay: Three-dimensional collagen lattices were prepared as previously described [30]. Briefly, type I collagen from rat tail (Sigma, Dorset, UK) was dissolved at 2 mg/ml in 0.1% acetic acid to create a stock solution. The collagen matrix was quickly prepared on ice by adding 6 ml of collagen stock solution to 3.6 ml of 0.1% acetic acid, 1.2 ml of 10× concentrated DMEM, and 1.2 ml of sodium bicarbonate solution (11.76 mg/ml) for a final concentration of 1 mg/ml collagen. The pH was adjusted to 7.2–7.4 by adding 0.1 mol/l sodium hydroxide solution. Fibroblasts were then added to achieve a final concentration of 5×10⁵ cells/ml of collagen suspension; 500 μl of this suspension was aliquoted into each well of a 24-well culture plate. After incubation for 15 min at 37 °C with 5% CO₂ for polymerization, the matrix in each well was overlayed with 500 μl of serum-free medium containing 100 ng/ml SPARC. The gels were gently mechanically released from the wall and bottom of the wells with a sterile spatula. Medium was changed every 3 days. Collagen lattices were scanned at various time points and lattice area was analyzed using the Digivision software (Soft Imaging System, Münster, Germany).

RNA isolation, cDNA generation, and northern blot analyses: After incubation, cells were washed two times with PBS and harvested from cell culture dishes in Trizol (Invitrogen) according to the manufacturer’s recommendations. The integrity of the total RNA was confirmed by gel electrophoresis. First-strand cDNA synthesis was prepared from total RNA using the Superscript cDNA Synthesis Kit (Invitrogen) according to manufacturer’s instructions.

A human SPARC cDNA fragment was amplified by PCR using the primer pairs 5’-TGC CTG ATG AGA CAG AGG TG-3’ and 5’-TAC AGG GTG ACC AGG ACG TT-3’, and cDNA of human Tenon’s fibroblasts as template (product length, 465 bp). PCR was performed in a final volume of 50 μl by initial denaturation at 94 °C for 2 min, followed by 35 cycles of 30 s at 94 °C, 45 s of annealing at 55 °C, and 90 s of extension at 72 °C. After the last cycle, the extension time was 10 min. PCR products were gel-purified by using the QIAEX II Gel Extraction Kit (QIAGEN, Hilden, Germany) and
cloned into pCRII-Topo vector (Invitrogen). After linearization of the vector with HindIII, antisense RNA probes for SPARC were generated and labeled with DIG-11-UTP using T7-polymerase (Roche, Mannheim, Germany).

For northern blot analysis, 10 µg of total RNA of human Tenon’s fibroblasts was separated on a 1% agarose gel containing 6% formaldehyde and blotted onto a positively charged nylon membrane (Roche). After transfer, the blot was cross-linked using a UV Stratalinker 1800 (Stratagene, La Jolla, CA). Prehybridization was performed for 1 h at 60 °C using the DIG EasyHyb-buffer (Roche). After overnight hybridization at 60 °C, membranes were washed for 5 min with 2× SSC and 0.1% SDS at room temperature and 15 min with 0.2× SSC and 0.1% SDS at 70 °C. For detection of hybridization signals, membranes were blocked for 30 min at room temperature in 1% blocking reagent, 0.1 M maleic acid, and 0.15 M NaCl, pH 7.5, and incubated 30 min in anti-digoxigenin-alkaline phosphatase diluted 1:10,000 (Roche). After washing membranes two times for 15 min in 0.1 M maleic acid, 0.15 M NaCl, pH 7.5, and 0.3% Tween-20, chemiluminescence detection was performed (CDP-Star; Roche). The membranes were exposed using a BAS 3000 Imager work station (Fujifilm, Düsseldorf, Germany). To monitor the integrity of RNA, the relative amounts of RNA loaded on the gel and the efficiency of transfer, membranes were stained with methylene blue. For densitometry AIDA Biopackage software (Raytest, Straubenhardt, Germany) was used.

Protein preparation and western blot analyses: For western blot analyses to detect SPARC, confluent human Tenon’s fibroblasts were starved overnight in serum free medium, and incubated with activated TGF-β1 or activated TGF-β2 (1 ng/ml; R&D Systems, Wiesbaden, Germany) for 3 days. The total cellular protein fraction was dissolved in radioimmunoprecipitation assay (RIPA) buffer and, after homogenization, insoluble constituents were removed by centrifugation. Protein content was measured by Bradford colorimetric assay according to manufacturers instructions (Thermo Scientific, Germany), and up to 25 µg of cytosolic proteins were subjected to a 10% SDS–PAGE. Separated proteins were transferred on a PVDF membrane (Roche) by semidry blotting. After blocking with 5% low fat milk in PBS-T, the membranes were incubated overnight with mouse-anti-SPARC antibodies (Santa Cruz), diluted 1:1,000 in PBS-T. After washing three times with PBS-T, membranes were incubated with HRP-conjugated goat-anti-mouse secondary antibodies at a 1:10,000 dilution in PBS-T (Millipore, Schwalbach, Germany). Antibody labeling was visualized using the Immobilon HRP substrate (Millipore), documented on a LAS 3000 Imager work station (Fujifilm, Düsseldorf, Germany) and quantified by using the AIDA Biopackage software (Raytest, Straubenhardt, Germany). As loading control and for quantification, a coomassie blue staining was used.

Immunohistochemistry: For immunohistochemistry, samples of three healthy-control and three scarred human Tenon’s capsules were fixed in 4% PFA for 4 h. After washing with 1x PBS overnight, samples were embedded in O.C.T compound (Sakura Finetek, Zoeterwoude, Netherlands) to process frozen sections according to standard protocols. Before overnight incubation at 4 °C with mouse monoclonal anti-β-SPARC antibodies (1:100; Santa Cruz, Santa Cruz, CA), samples were incubated in 3% BSA (BSA) solution for 30 min. As negative control, sections were incubated with 3% BSA in PBS overnight. After three washes (10 min each) with PBS, samples were treated for 1 h with Alexa 488 Fluor-labeled goat-anti-mouse antibodies (1:1,000; Invitrogen). After three washes, sections were incubated in a 1:10,000 propidium iodide solution (stock 0.33 mg/ml in PBS) for 15 min, washed three additional times and mounted in fluorescent mounting medium. Samples were analyzed on an Axiovision fluorescence microscope (Carl Zeiss, Jena, Germany).

Statistics: All results are expressed as mean±SEM. Comparisons between the mean variables of 2 groups were made by a 2-tailed Student’s t-test. P values less than 0.05 were considered to be statistically significant.

RESULTS

SPARC expression is increased in human Tenon’s capsule scars: To analyze if SPARC is expressed in scars of the Tenon’s capsule after filtration surgery, immunohistochemical staining using specific antibodies against SPARC in healthy and scarred human Tenon’s capsule was performed.

In the normal human Tenon’s capsule, a weak but specific signal for SPARC was only detected around and within blood vessel walls (Figure 1B). Similar to this observation, in the scarred human Tenon’s capsule, SPARC was also localized around and within blood vessel walls, but the staining intensity was markedly increased when compared to healthy specimens (Figure 1C). Additionally, the blood vessels showed a vasodilation in the scarred human Tenon’s capsule, which is typical for scar tissues. In the normal human Tenon’s capsule, only a weak signal for SPARC was detected within the connective tissue, whereas in scars of the Tenon’s capsule a specific staining was observed throughout the collagenous stroma (Figure 1). In the extracellular matrix of human Tenon’s scars, a prominent localization of SPARC was seen in areas of a denser connective tissue (Figure 1C, arrow heads).

TGF-β induces the expression of SPARC in human Tenon’s fibroblasts: After filtration surgery, increased levels of TGF-β are a major risk factor for the formation of Tenon’s capsule scars. Therefore we wondered if TGF-β1 and/or TGF-β2 can induce the expression of SPARC in cultured HTF cells.
Untreated HTF cells showed only weak expression for \textit{SPARC} mRNA. After incubation of the cells with activated TGF-\(\beta\)-1 (1 ng/ml) and -\(\beta\)-2 (1 ng/ml) for three days, a marked increased mRNA expression of \textit{SPARC} was observed by northern blot analyses when compared with untreated control cells. In addition, the treatment of HTF cells with TGF-\(\beta\)-1 caused an 18.9 fold induction of \textit{SPARC} mRNA and was more potent as that with TGF-\(\beta\)-2 that mediates a 10.6 fold increase (Figure 2A-C).

To investigate whether the increase in transcription of \textit{SPARC} mRNA also results in elevated protein level, western blot analyses were performed. In untreated control cells, a basal protein level of \textit{SPARC} expression was detected, whereas the TGF-\(\beta\)-1 (1 ng/ml) and TGF-\(\beta\)-2 (1 ng/ml) treatments lead to a higher synthesis rate in HTF cells. Similar to northern blot analyses, we could also observe higher protein levels after treatment of HTF cells with TGF-\(\beta\)-1 (3.6 fold) as that after incubation with TGF-\(\beta\)-2 (2.1 fold), although it was not such prominent like on the mRNA level. Again, TGF-\(\beta\)-1 was more potent than TGF-\(\beta\)-2 to induce \textit{SPARC} protein synthesis (Figure 2D-F).

\textit{SPARC} induces proliferation of HTF cells: During wound healing fibroblast cell proliferation is commonly involved in the process of scar formation. To investigate if \textit{SPARC} can
promote these processes, HTF cells were incubated with different concentrations of SPARC and cell proliferation was analyzed by BrdU-ELISA assays.

After an incubation of HTF cells with 1 ng/ml SPARC for three days, only a slight increase of cell proliferation was detected, whereas higher concentrations of SPARC significantly induced HTF cell proliferation in a dose dependent manner up to 1.6 fold when compared to untreated controls (Figure 3A).

Because TGF-β1 is a very potent inducer of HTF cell proliferation and also of SPARC expression, we analyzed whether SPARC-mediated HTF cell proliferation is a downstream effect of TGF-β1. Since SPARC can activate the TGF-β signaling cascade [27] and HTF cells express TGF-β1 [31], only moderate concentrations of both factors were used to avoid an overstimulation.

After an incubation of HTF cells with 200 pg/ml activated TGF-β1 for 3 days a statistically significant increase of cell proliferation of about 1.7 fold was observed in comparison to untreated controls. In contrast, only a moderate reduction in relative collagen lattice size was observed in untreated control cells over time. These data strongly indicate a substantial contractile effect of SPARC on HTF cells.

DISCUSSION

In the present study, we demonstrated that SPARC is present in healthy human Tenon’s capsule, where it was restricted to blood vessels, in agreement with findings in other tissues [32]. In scarred human Tenon’s capsules a more intense SPARC staining was observed in blood vessel walls and especially throughout the connective tissue. The higher concentration of SPARC within this tissue could lead to enhanced matricellular effect of SPARC within the Tenon’s capsules.

These findings indicate that SPARC is highly expressed in scarred human Tenon’s capsule and that Tenon’s capsule fibroblasts could be the source of increased SPARC synthesis.

Activation of HTF cells by cytokines and growth factors is considered to be the key effect during the subconjunctival wound healing response [33]. We found that SPARC expression is significantly increased in HTF cells after treatment with TGF-β1 and TGF-β2. Despite the fact that both TGF-β isoforms provoke a higher amount of SPARC as compared to untreated basal levels, we found that TGF-β1 lead to a higher increase of SPARC synthesis compared to TGF-β2. In addition, TGF-β-mediated induction of SPARC mRNA was much more pronounced than that of intracellular SPARC proteins. This alleged discrepancy could be explained by different half-lives of SPARC mRNA and protein, and by the circumstance that SPARC is secreted and associated with the extracellular matrix.

In the anterior chamber and especially in the AH of normal eyes, TGF-β2 is the predominant TGF-β isoform. Both TGF-β1 and TGF-β2 isoforms are elevated in different types of glaucoma. The concentration of latent/active TGF-β1 is
increased in the AH of patients with pseudoexfoliation glaucoma (PEXG) [34], whereas latent/active TGF-β2 is elevated in the AH of patients with primary open-angle glaucoma (POAG). In POAG patients failure of the filtering bleb after surgery is correlated with increased preoperative TGF-β2 levels in AH [35]. Further, Nguyen et al. [36] demonstrated that there is a substantial risk of a breakdown of blood-aqueous barrier after trabeculectomy, which could also contribute to an additional increase of the TGF-β concentration within the AH, especially of TGF-β1, the dominant isoform in the blood. Since TGF-β1 and TGF-β2 are potent factors contributing to an activation of fibroblasts throughout the body, increased amounts of TGF-β1 and TGF-β2 in the AH could be major risk factors leading to a failure of glaucoma filtration surgery by activating HTF cells. The observed increase of SPARC expression within the scarring tissue of the Tenon’s capsule could be mediated by TGF-β.

The activating capability of TGF-β1 and TGF-β2 on HTF cell functions was previously demonstrated by numerous studies. The TGF-β induced activation of HTF cells resulted in an enhanced proliferation rate, an increased migration rate and stronger collagen gel contraction. Our findings showed that HTF proliferation can be also stimulated by SPARC, although its effect was lower than that of TGF-β. However a combined treatment with SPARC and TGF-β1 showed an additive effect suggesting that SPARC contributes to the activation of HTF cells. Since basal and TGF-β-mediated expression of extracellular matrix components is increased in HTF cells of patients having PEX glaucoma or POAG [29], it is of particular interest if this is also true for SPARC expression and hereby a TGF-β independent scarring response could be mediated.

Although the molecular mechanisms of SPARC signaling are still unknown, different studies showed that SPARC treatment provokes activation of latent TGF-β [27, 28]. Consistently, an enhanced phosphorylation of Smad2 was observed after SPARC treatment [37], showing that the increase in active TGF-β also led to an enhanced TGF-β signaling. Therefore the additive effect of SPARC on TGF-β-mediated cell proliferation might be also partially due to an enhanced activation of TGF-β.

In addition to increased proliferation, activated fibroblasts mediate contraction of connective tissue constituting another critical step in scar tissue formation. An assay to test whether a substance is contributing to this aspect of the wound healing process is a collagen gel contraction assay. We showed that SPARC can stimulate contraction of collagen gels by HTF cells and thereby contribute to the scarring process.

SPARC is known to be a collagen-binding matricellular protein and its expression is found to be elevated during wound healing processes [38] and in tissues with fibrotic disorders [12,20,39-43]. The high affinity binding site of SPARC for collagen type I, type III, and type IV is localized.
in the third domain of the SPARC protein and contains two high affinity Ca^2+-binding EF hands [44]. The interaction between SPARC and collagen suggests that SPARC plays an important role during ECM assembly. This assumption was supported by the finding that the skin of SPARC−/− mice has approximately half the amount of collagen as compared to wild type littermates [45]. A decrease in collagen was also apparent in the heart and in fat deposits of SPARC−/− mice [46,47]. Therefore, an increased amount of SPARC within Tenon capsule together with higher amounts of TGF-β, which is known to increase matrix production in HTF cells, could amplify the scarring process in the Tenon’s capsule following filtration surgery. In accordance with this, in the SPARC−/− mice, a pivotal role of SPARC in the organization of the extracellular matrix in the surgically induced fistula was demonstrated. In these mice a reduced post-operative subconjunctival scarring reaction was observed [26].

In summary, our data reveals for the first time that SPARC is highly expressed in scarred human Tenon’s capsules in vivo and that SPARC can directly induce and amplify the effect of TGF-β on HTF cell proliferation and collagen gel contraction in vitro. Together with observations in SPARC−/− mice after filtration surgery it is tempting to speculate that a modulation of SPARC expression could be a feasible therapeutic approach to reduce excessive scarring and subsequently to prevent failure of glaucoma filtration surgery.

ACKNOWLEDGMENTS

The study was supported through grants from the ELAN program of the University of Erlangen-Nürnberg, Erlangen, Germany. The authors would like to thank Jasmine Onderka, Elke Meyer and Angelika Pach for excellent technical assistance.

REFERENCES

1. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 2006; 90:262-7. [PMID: 16488940]
2. Kass MAHeuerDKHigginbothamEJJohnsonCAKeltnerJLMill erJPParrishRKIIWilsonMRGordonMOfor theOcular Hypertension Treatment Study Group. The Ocular Hypertension Treatment Study: A Randomized Trial Determines That Topical Ocular Hypotensive Medication Delays or Prevents the Onset of Primary Open-Angle Glaucoma. Arch Ophthalmol200212070113 [PubMed: 12049574]
3. Khaw PT, Ocleonst NL, Schultz G, Grierson I, Sherwood MB, Larkin G. Activation and suppression of fibroblast function. Eye 1994; 8:188-95. [PMID: 7958020]
4. The Fluorouracil Filtering Surgery Study Group. Five-year follow-up of the Fluorouracil Filtering Surgery Study. Am J Ophthalmol 1996; 121:349-66. [PMID: 8604728]
5. Katz GJ, Higginbotham EJ, Lichter PR, Skuta GL, Musch DC, Bergstrom TJ, Johnson AT. Mitomycin C versus 5-fluorouracil in high-risk glaucoma filtering surgery. Extented follow-up. Ophthalmology 1995; 102:1263-9. [PMID: 9097762]
6. Yoon PS, Singh K. Update on antifibrotic use in glaucoma surgery, including use in trabeculectomy and glaucoma drainage implants and combined cataract and glaucoma surgery. Curr Opin Ophthalmol 2004; 15:141-6. [PMID: 15021227]
7. Daniels JT, Ocleonst NL, Crowston JG, Cordeiro MF, Alexander RA, Wilkins M, Porter R, Brown R, Khaw PT. Understanding and controlling the scarring response: The contribution of histology and microscopy. Microse Res Tech 1998; 42:317-33. [PMID: 9766427]
8. Cordeiro MF, Bhattacharya SS, Schultz GS, Khaw PT. TGF-{beta}1, -{beta}2, and -{beta}3 In Vitro: Biphasic Effects on Tenon’s Fibroblast Contraction, Proliferation, and Migration. Invest Ophthalmol Vis Sci 2000; 41:756-63. [PMID: 10711691]
9. Costa VP, Spaeth GL, Eiferman RA. S O-N. Wound healing modulation in glaucoma filtration surgery. Ophthalmic Surg 1993; 24:152-70. [PMID: 8483566]
10. Khaw PT, Chang L, Wong TTL, Mead A, Daniels JT, Cordeiro MF. Modification of wound healing after glaucoma surgery. Curr Opin Ophthalmol 2001; 12:143-8. [PMID: 11224722]
11. Bornstein P, Sage EH. Matricellular proteins: extracellular modulators of cell function. Curr Opin Cell Biol 2002; 14:608-16. [PMID: 12231357]
12. Pichler RH, Hugo C, Shankland SJ, Reed MJ, Bassuk JA, Andoh TF, Lombardi DM, Schwartz SM, Bennett WM, Alpers CE, Sage EH, Johnson RJ, Couser WG. SPARC is expressed in renal interstitial fibrosis and in renal vascular injury. Kidney Int 1996; 50:1978-89. [PMID: 8943481]
13. Francki A, Sage EH. SPARC and the Kidney Glomerulus: Matricellular Proteins Exhibit Diverse Functions under Normal and Pathological Conditions. Trends Cardiovasc Med 2001; 11:32-7. [PMID: 11413050]
14. Chang W, Wei K, Jacobs SS, Upadhyay D, Weil D, Rosen GD. SPARC Suppresses Apoptosis of Idiopathic Pulmonary Fibrosis Fibroblasts through Constitutive Activation of β2-Catenin. J Biol Chem 2010; 285:8196-206. [PMID: 20061390]
15. Frizzell E, Liu SL, Abraham A, Ozaki I, Eghbali M, Sage EH, Zern MA. Expression of SPARC in normal and fibrotic livers. W.B. Saunders; 1995. p. 84–57.
16. Nakatani K, Seki S, Kawada N, Kitada T, Yamada T, Sakaguchi H, Kadoya H, Ikeda K, Kanaeda K. Expression of SPARC by activated hepatic stellate cells and its correlation with the stages of fibrogenesis in human chronic hepatitis. Virchows Arch 2002; 441:466-74. [PMID: 12447677]
17. Berryhill BL, Kane B, Stramer B, Fini EM, Hassell JR. Increased SPARC accumulation during corneal repair. Exp Eye Res 2003; 77:85-92. [PMID: 12823991]
18. Mishima H, Hibino T, Hara H, Murakami J, Otori T. SPARC from corneal epithelial cells modulates collagen contraction by keratocytes. Invest Ophthalmol Vis Sci 1998; 39:2547-53. [PMID: 9856764]
19. Macko RF, Gelber AC, Young BA, Lowitt MH, White B, Wigley FM, Goldblum SE. Increased circulating concentrations of the counteradhesive proteins SPARC and thrombospondin-1 in systemic sclerosis (scleroderma). Relationship to platelet and endothelial cell activation. J Rheumatol 2002; 29:2565-70. [PMID: 12465153]
20. Zhou X, Tan FK, Reveille JD, Wallis D, Milewicz DM, Ahn C, Wang A, Arnett FC. Association of novel polymorphisms with the expression of SPARC in normal fibroblasts and with susceptibility to scleroderma. Arthritis Rheum 2002; 46:2990-9. [PMID: 12428242]

21. Strandjord TP, Madtes D, Weiss D, Sage E. Collagen accumulation is decreased in SPARC-null mice with bleomycin-induced pulmonary fibrosis. Am J Physiol 1999; 277:L628-35. [PMID: 10844771]

22. Socha MJ, Manhiani M, Said N, Imig J, Motamed K. Secreted protein acidic and rich in cysteine deficiency ameliorates renal inflammation and fibrosis in angiotensin hypertension. Am J Pathol 2007; 171:1104-12. [PMID: 17717147]

23. Wang J-C, Lai S, Guo X, Zhang X, de Crombrugghe B, Sonnynlal S, Arnett F, Zhou X. Attenuation of fibrosis in vitro and in vivo with SPARC siRNA. Arthritis Res Ther 2010; 12:R60. [PMID: 20359365]

24. Camino AM, Atorrasagasti C, Maccio D, Prada F, Salvatierra E, Rizzo M, Alaniz L, Aquino JB, Podhajcer OL, Silva M, Mazzolini G. Adenovirus-mediated inhibition of SPARC attenuates liver fibrosis in rats. J Gene Med 2008; 10:993-1004. [PMID: 18615449]

25. Bradshaw AD, Reed MJ, Sage EH. SPARC-null Mice Exhibit Accelerated Cutaneous Wound Closure. J Histochem Cytochem 2002; 50:1-10. [PMID: 11748289]

26. Seet L-F, Su R, Barathi VA, Lee WS, Poh R, Heng YM, Manser E, Vithana EN, Aung T, Weaver M, Sage EH, Wong TT. SPARC Deficiency Results in Improved Surgical Survival in a Novel Mouse Model of Glaucoma Filtration Surgery. PLoS ONE 2010; 5:e9415. [PMID: 20195533]

27. Francki A, McClure TD, Brekken RA, Motamed K, Murri C, Wang T, Sage EH. SPARC regulates TGF-beta-dependent signaling in primary glomerular mesangial cells. J Cell Biochem 2004; 91:915-25. [PMID: 15034927]

28. Schemmann BJ, Neil JR, Schemmann WP. SPARC Inhibits Epithelial Cell Proliferation in Part through Stimulation of the Transforming Growth Factor-β-Signaling System. Mol Biol Cell 2003; 14:3977-88. [PMID: 14517312]

29. Kottler UB, Jüinemann AGM, Aigner T, Zenkel M, Rummelt C, Schlötzer-Schrehardt U. Comparative effects of TGF-β1 and TGF-β2 on extracellular matrix production, proliferation, migration, and collagen contraction of human dermal fibroblasts in pulmonary fibrosis and experimental primary open-angle glaucoma. Exp Eye Res 2005; 80:121-34. [PMID: 15652533]

30. Pena RA, Jerdan J, Glaser B. Effects of TGF-beta and TGF-beta neutralizing antibodies on fibroblast- induced collagen gel contraction: implications for proliferative vitreoretinopathy. Invest Ophthalmol Vis Sci 1994; 35:2804-8. [PMID: 8188474]

31. Tripathi RC, Li J, Chalam KV, Tripathi BJ. Expression of Growth Factor mRNAs by Human Tenon’s Capsule Fibroblasts. Exp Eye Res 1996; 63:339-46. [PMID: 8943707]

32. Jandeleit-Dahm K, Rumble J, Cox AJ, Kelly DJ, Dziadek M, Cooper ME, Gilbert RE. SPARC Gene Expression Is Increased in Diabetes-Related Mesenteric Vascular Hypertrophy. Microvasc Res 2000; 59:61-71. [PMID: 10625572]

33. Cordeiro MF. Role of transforming growth factor beta in conjunctival scarring. Clin Sci 2003; 104:181-7. [PMID: 12546640]

34. Schlötzer-Schrehardt U, Zenkel M, Küchle M, Sakai LY, Naumann GOH. Role of Transforming Growth Factor-[β1] and its Latent Form Binding Protein in Pseudoexfoliation Syndrome. Exp Eye Res 2001; 73:765-80. [PMID: 11846508]

35. Pich G, Welge-Luessen U, Grehn F, Lütjen-Drecoll E. Transforming growth factor β2 levels in the aqueous humor in different types of glaucoma and the relation to filtering bleb development. Graefes Arch Clin Exp Ophthalmol 2001; 239:199-207. [PMID: 11405069]

36. Nguyen NX, Küchel M, Martus P, Naumann GOH. Quantification of Blood-Aqueous Barrier Breakdown after Trabeculectomy: Pseudoexfoliation versus Primary Open-Angle Glaucoma. J Glaucoma 1999; 8:18-23. [PMID: 10084270]

37. Schellings MWM, Vanhoutte D, Swinnen M, Cleutjens JP, Debets J, van LeeuwenREW, d’Hooge J, Van de Werf F, Carmeliet P, Pinto YM, Sage EH, Heymans S. Absence of SPARC results in increased cardiac rupture and dysfunction after acute myocardial infarction. J Exp Med 2009; 206:113-23. [PMID: 19103879]

38. Bradshaw AD, Sage EH. SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. J Clin Invest 2001; 107:1049-54. [PMID: 11342565]

39. Frizzell E, Liu SL, Abraham A, Ozaki I, Eghbali M, Sage EH, Zern MA. Expression of SPARC in normal and fibrotic livers. Hepatology 1995; 21:847-54. [PMID: 7875683]

40. Kanauchi M, Nishioka M, Dohi K. Secreted protein acidic and rich in cysteine deficiency ameliorates bleomycin-induced pulmonary fibrosis. Am J Physiol 1999; 277:L628-35. [PMID: 10484471]

41. Kuhn C, Mason R. Immunolocalization of SPARC, tenasin, and thrombospondin in pulmonary fibrosis. Am J Pathol 1995; 147:1759-69. [PMID: 7495300]

42. Pichler RH, Bassuk JA, Hugo C, Reed MJ, Eng E, Gordon KL, Pippin J, Alpers CE, Couser WG, Sage EH, Johnson RJ. SPARC is expressed by mesangial cells in experimental mesangial proliferative nephritis and inhibits platelet-derived-growth-factor-medicated mesangial cell proliferation in vitro. Am J Pathol 1996; 148:1153-67. [PMID: 8644597]

43. Zhou X, Tan F, Guo X, Arnett F. Attenuation of collagen synthesis with small interfering RNA of SPARC in cultured fibroblasts from the skin of patients with scleroderma. Arthritis Rheum 2006; 54:2626-31. [PMID: 16871529]

44. Hohenester E, Maurer P, Timpl R. Crystal structure of a pair of follistatin-like and EF-hand calcium-binding domains in BM-40. EMBO J 1997; 16:3778-86. [PMID: 9233787]

45. Bradshaw AD, Paolakkainen P, Dasgupta J, Davidson JM, Wight TN, Sage EH. SPARC-Null Mice Display Abnormalities in the Dermis Characterized by Decreased Collagen Fiber Diameter and Reduced Tensile Strength. J Invest Dermatol 2003; 120:949-55. [PMID: 12787119]

46. Bradshaw AD, Graves DC, Motamed K, Sage EH. SPARC-null mice exhibit increased adiposity without significant differences in overall body weight. Proc Natl Acad Sci USA 2003; 100:6045-50. [PMID: 12721366]
47. Bradshaw AD, Baicu CF, Rentz TJ, Van Laer AO, Boggs J, Lacy JM, Zile MR. Pressure Overload-Induced Alterations in Fibrillar Collagen Content and Myocardial Diastolic Function: Role of Secreted Protein Acidic and Rich in Cysteine (SPARC) in Post-Synthetic Procollagen Processing. Circulation 2009; 119:269-80. [PMID: 19118257]