Matricellular Protein Levels in Aqueous Humor and Surgical Outcomes of Trabeculectomy

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In China, primary angle closure is estimated to cause unilateral blindness in 1.5 million individuals and bilateral blindness in another 1.5 million.1 Out of all forms of angle closure, acute primary angle closure (APAC) is regarded as an ophthalmic emergency, which is characterized by clinical signs and symptoms including rapid and excessive intraocular pressure (IOP) increase, corneal edema, a mid-dilated pupil, vascular congestion, eye pain, headache, nausea, and/or vomiting.1 After the acute angle closure is broken, some APAC patients will still have considerable peripheral anterior synechiae and uncontrolled IOP and filtration surgery will then be performed.

Glaucoma filtration surgery attempts to control IOP by creating an artificial drainage route from the anterior chamber to the subconjunctival space, which can be defined as a surgical wound.3 Excessive extracellular matrix (ECM) production and scar formation will close the generated passage for the aqueous humor, leading to failure of the surgery.3

In a previous study, we quantified levels of matricellular proteins in aqueous humor samples from APAC and non-glaucomatous cataract eyes. Increased levels of secreted protein acidic and rich in cysteine (SPARC), thrombospondin-2, and osteopontin—were measured using multiplexed immunoassays kits. Intraocular pressure was measured using Goldmann application tonometry. Bleb morphology was assessed using anterior segment optical coherence tomography, and bleb score was calculated according to bleb size and reflectivity.

When previous APAC eyes were divided according to surgical outcome 18 months after trabeculectomy, SPARC protein was significantly higher in aqueous humor in the failure group (P = 0.009). When previous APAC eyes were divided according to SPARC level, eyes with low SPARC levels had significantly higher overall success rate compared with eyes with high SPARC levels (P = 0.005 for complete success and P = 0.018 for qualified success). Multiple logistic regression analyses showed that eyes with higher levels of SPARC were more likely to have a failed filtration surgery than were eyes with lower levels of SPARC. For complete success, P = 0.006 and odds ratio (OR) = 6.458; for qualified success, P = 0.033 and OR = 2.608. The level of SPARC was found to have a positive correlation with bleb score (P < 0.001, R2 = 0.471).

CONCLUSIONS. In previous APAC patients, the SPARC level in aqueous humor is a prognostic factor for surgical results of trabeculectomy. Modulation of SPARC expression may have potential clinical applications after filtration surgery.

Keywords: matricellular proteins, SPARC, trabeculectomy
with the Declaration of Helsinki. Subjects included were previous APAC patients who had had an APAC attack within 1 month before admission to our hospital. Patients still had uncontrolled IOP under standardized antiglaucomatous medications after pupil block was released. All patients agreed to receive trabeculectomy to reduce IOP. Participants were recruited prospectively and consecutively at Shanghai General Hospital from May 2016 to November 2016. Exclusion criteria were (1) eyes with ocular diseases other than APAC, (2) eyes with a history of intraocular incisional surgery, and (3) eyes that had an IOP value of <21 mm Hg at all three visits prior to surgery. The standard medication treatment for previous APAC is listed below. Some or all of the following were used according to the patient’s IOP and physical status: (1) topical pilocarpine 1% four times daily, (2) topical β-adrenergic antagonists two times daily, (3) topical α2-adrenergic agonists two times daily, (3) topical steroids four times daily, and (4) topical and/or oral carbonic anhydrase inhibitors.

Surgical Technique and Postoperative Care

Trabeculectomy was performed by one surgeon (MF). After making a fornix-based conjunctival flap and dissecting a limbus-based 4×4-mm scleral flap, a 5-fluorouracil-soaked (25 mg/mL) sponge was placed underneath the conjunctival flap for 3 minutes. Afterward, 250 mL balanced salt solution was used to wash the surgical area. A trabeculectomy and a basal iridectomy were then performed. The scleral flap was sutured with two 10-0 nylon sutures at its corners, and the centers of two sides were closed with two adjustable sutures to allow minimal leakage during reformation of the anterior chamber. Finally, the conjunctiva was closed with 10-0 nylon suture.

A topical antibiotic and a topical nonsteroidal anti-inflammatory medication were given four times daily for 1 week. Topical corticosteroids were used every 2 to 3 hours for the first week, and then tapered over the next 1 to 2 months. No antimetabolites were used during follow-up. In case of postoperative IOP measurements > 21 mm Hg, despite ocular massage and loosening of adjustable sutures, IOP-lowering medication was added. In case of still inadequate IOP control, additional surgical procedures could be performed as required.

Collection of Aqueous Humor

Briefly, aqueous humor samples (50–100 μL) were collected at the very beginning of trabeculectomy through limbal paracentesis. Samples were immediately stored in liquid nitrogen and transferred to −80°C until they were analyzed.

Measurement of Cytokine Levels in Aqueous Humor

Three matricellular proteins, SPARC, TSP-2, and osteopontin, were detected using an assay (Luminex Screening Human Magnetic Assay; R&D Systems, Inc., Minneapolis, MN, USA). The assay was performed according to the manufacturer’s instructions. Aqueous humor samples were diluted 1:2 for the assay, and 50 μL of the diluted samples was added to each well. Fluorescence intensity was acquired and analyzed using software (Luminex xponent3.1; Luminex, Austin, TX, USA).

Outcome Measures

The preoperative IOP and all topical antiglaucomatous medications used were recorded before surgery. Patients were followed up at 1 week and 1, 3, 6, 12, and 18 months. Goldmann applanation tonometry and slit-lamp biomicroscopy were performed at each postoperative visit. The number of antiglaucomatous medications used and any additional surgeries performed were also recorded. We defined surgical failure as an IOP value ≥21 mm Hg or if any additional glaucoma surgeries were required. If IOP of one visit could be controlled lower than 21 mm Hg without the use of topical antiglaucomatous medication, the visit was defined as “complete success.” If IOP of one visit was <21 mm Hg with eye drops, the visit was defined as a “qualified success.” For instance, a patient who had an IOP (without eye drops) of 24 mm Hg on one follow-up and 18 mm Hg (with eye drops) at the subsequent visit was defined as “qualified success” at the second visit.

Anterior segment optical coherence tomography (AS-OCT) images of the bleb were taken at postoperative month 18 with an imaging system (CASIA SS-1000; Tomey, Nagoya, Japan). An 8×8-mm scan of each bleb, including the bleb apex, was acquired. Scoring was carried out according to the novel grading system created by Wen et al. A scale of 0 to 3 using 0.5 increments was applied to the image through the apex of the bleb by comparing it to the reference photographs. Larger blebs with greater bleb height and width and with lower reflectivity were given lower grades. When the size and the reflectivity of blebs were not parallel, the thickness and reflectivity of the bleb wall was evaluated. Encapsulated blebs tend to have a scarred wall with high reflectivity. Thus, a hyporeflective thick bleb wall with multiple parallel layers or cyst, suggesting the bleb was functional, was given lower grade. The score of a bleb was the mean value of scores given by two experienced graders. An additional grader was assigned to reevaluate the images and gave the final score if there was too much discrepancy (>1) between the two separated graders.

Statistical Analysis

Commercially available statistical analysis software (SPSS version 22.0; SPSS, Inc., Chicago, IL, USA) was used for the analyses. Continuous variables (e.g., levels of matricellular proteins) were expressed as mean and standard deviation. Categorical variables (e.g., sex) were expressed as number and frequency. Independent Student’s t-tests were applied to compare data between two groups. Pearson χ² test was used for categorical data. Binary logistic regressions were used to assess the relationship between the clinical outcome and matricellular protein levels in the aqueous humor. If any matricellular protein was detected to be a significant risk factor for trabeculectomy failure, we divided the patients into two groups according to the level of this protein, and Kaplan Meyer survival curves were used to display the success rate of end points over time between groups. Linear regressions were used to assess the relationship between the matricellular protein levels and bleb score assessed by AS-OCT. The coefficient of determination R² was used to express the proportion of the variation in the dependent variable explained by the regression model. The level of statistical significance was set at P < 0.05.

RESULTS

Patients

In total, 40 previous APAC patients were included in this study. The clinical characteristics are shown in Table 1. The mean (SD) levels (ng/mL) of SPARC, TSP-2, and osteopontin in aqueous humor samples were 2.938 (1.159), 0.245 (0.408), and 18.382 (14.412), respectively. All patients finished the 18 months of follow-up. During the follow-up period, 29 eyes (72.5%) were classified into the complete success group and 11 eyes (27.5%) into the failure group. There were no
significant differences between both groups considering age, sex, preoperative IOP, numbers of preoperative medications, and the interval between the onset of APAC and trabeculectomy. However, the mean levels of SPARC and osteopontin were significantly higher in the 11 failed eyes compared to the 29 complete successful eyes ($P = 0.009$ and $0.004$, respectively) (Table 1).

### Correlation Between Matricellular Proteins and Surgical Results

The preoperative IOP and levels of SPARC, TSP-2, and osteopontin were included in the multiple logistic regression analysis. Considering the matricellular protein SPARC, eyes with higher levels of SPARC were more likely to have a failed filtration surgery than were eyes with lower levels of SPARC. For complete success, $P = 0.006$, odds ratio (OR) = 6.458, and 95% confidence interval (CI) = 1.728 to 24.133; for qualified success, $P = 0.033$, OR = 2.638, and 95% CI = 1.081 to 6.292. The other factors, including preoperative IOP and levels of TSP-2 and osteopontin, were not associated with the outcome of trabeculectomy. Results indicated that high SPARC level was a significant risk factor for trabeculectomy failure.

We subsequently divided the 40 previous APAC patients into two groups according to the level of SPARC. Twenty eyes with a SPARC level lower than the median value (<2.638 ng/mL) were included in group L, and 20 patients with SPARC level higher than the median value (>2.638 ng/mL) were included in group H. The postoperative outcomes including postoperative IOP, antiglaucomatous eye drop usage, and success rates are summarized in Table 2. The complete success rates at 18 months after trabeculectomy were 90% and 55% in group L and group H, respectively. The qualified success rates at 18 months after trabeculectomy were 100% and 75% in group L and group H, respectively. A Wilcoxon test revealed that the differences in success rates at 18 months between both groups were significant ($P = 0.013$ for complete success, and $P = 0.047$ for qualified success). The Kaplan-Meier survival plots were drawn to investigate the overall success rate between both groups (Fig. 1). Eyes with low SPARC levels (group L) had significantly higher complete success rate and qualified success rate over the 18 months of follow-up compared to eyes with high SPARC levels (group H) ($P = 0.005$ and $P = 0.018$, respectively) (Fig. 1).

### Correlation Between Matricellular Proteins and Bleb Scores

The relationship between levels of SPARC, TSP-2, and osteopontin with bleb scores at postoperative 18 months was evaluated (Fig. 2). Only the SPARC level had significantly positive correlation with bleb score ($P < 0.001$, $R^2 = 0.471$), for which the bleb score ($y$) was related to the SPARC level ($x$) according to the formula $y = 0.545 + 0.408x$.

### DISCUSSION

Trabeculectomy is the gold standard in surgical management of medically uncontrolled glaucoma. The poor outcome is usually due to the fibrosis of the surgical site. One possible explanation for the enhanced fibrosis is the overexpression of related proteins.

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**Table 1.** Characteristics of 40 Previous APAC Patients Included

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Previous APAC</th>
<th>Successful Group</th>
<th>Failed Group</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, no.</td>
<td>40</td>
<td>29</td>
<td>11</td>
<td>0.235</td>
</tr>
<tr>
<td>Male/female</td>
<td>15/25</td>
<td>13/16</td>
<td>2/9</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>68 ± 11</td>
<td>69 ± 10</td>
<td>66 ± 13</td>
<td>0.454</td>
</tr>
<tr>
<td>Preoperative IOP</td>
<td>33.1 ± 9.0</td>
<td>31.5 ± 8.1</td>
<td>37.4 ± 10.2</td>
<td>0.066</td>
</tr>
<tr>
<td>Number of antiglaucoma eyedrops</td>
<td>2.3 ± 1.1</td>
<td>2.3 ± 1.1</td>
<td>2.3 ± 1.1</td>
<td>0.094</td>
</tr>
<tr>
<td>Interval between the onset of APAC and trabeculectomy, d</td>
<td>15.7 ± 9.1</td>
<td>15.2 ± 9.0</td>
<td>17.0 ± 9.6</td>
<td>0.575</td>
</tr>
<tr>
<td>SPARC, ng/mL</td>
<td>2.938 ± 1.159</td>
<td>2.525 ± 0.639</td>
<td>4.025 ± 1.517</td>
<td>0.009*</td>
</tr>
<tr>
<td>Osteopontin, ng/mL</td>
<td>0.245 ± 0.408</td>
<td>0.146 ± 0.225</td>
<td>0.505 ± 0.636</td>
<td>0.094</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>18.382 ± 14.442</td>
<td>14.439 ± 11.720</td>
<td>28.777 ± 16.276</td>
<td>0.004*</td>
</tr>
</tbody>
</table>

* $P < 0.01$.

**Table 2.** Surgical Results Between Groups With Low Levels of SPARC and High Levels of SPARC

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Previous APAC With Low Levels of SPARC, Group A</th>
<th>Previous APAC With High Levels of SPARC, Group B</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, no.</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>SPARC level, ng/mL</td>
<td>2.148 ± 0.379</td>
<td>3.727 ± 1.141</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Probability of complete success, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>95</td>
<td>75</td>
<td>0.182</td>
</tr>
<tr>
<td>12 months</td>
<td>90</td>
<td>65</td>
<td>0.127</td>
</tr>
<tr>
<td>18 months</td>
<td>90</td>
<td>55</td>
<td>0.013*</td>
</tr>
<tr>
<td>Probability of qualified success, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>100</td>
<td>95</td>
<td>1.0</td>
</tr>
<tr>
<td>12 months</td>
<td>100</td>
<td>85</td>
<td>0.231</td>
</tr>
<tr>
<td>18 months</td>
<td>100</td>
<td>75</td>
<td>0.047*</td>
</tr>
<tr>
<td>IOP at 18 months postoperatively</td>
<td>13.9 ± 2.5</td>
<td>15.2 ± 2.4</td>
<td>0.115</td>
</tr>
<tr>
<td>Number of glaucoma eyedrops at 18 months postoperatively</td>
<td>0.1 ± 0.3 (0-1)</td>
<td>0.4 ± 0.8 (0-2)</td>
<td>0.195</td>
</tr>
</tbody>
</table>

* $P < 0.05$. 

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Matricellular Proteins and Trabeculectomy Outcome

Fibrosis is regulated by series of factors, of which the transforming growth factor-β (TGF-β) family has drawn the most attention. However, the CAT-1520120 Trabeculectomy Study Group used a monoclonal antibody to TGF-β to prevent scarring after the first trabeculectomy, and they showed no difference between treatment and placebo groups in preventing the failure of primary trabeculectomy. It is conceivable that directing against TGF-β is too narrow an approach in humans. Other factors besides TGF-β are also involved, as are other factors that may mediate the effects of TGF and scar formation.

Matricellular proteins were first proposed in 1995 to describe a group of nonstructural secreted glycoproteins that enable cells to communicate with and control their surrounding ECM. The original family includes SPARC, TSP-1 and -2, tenascin-C and -X, osteopontin, and other proteins. They were found widely expressed in all kinds of glaucomatous eyes, were found widely expressed in all kinds of glaucomatous eyes, and the overexpression of these proteins was noted to add in preventing the failure of primary trabeculectomy. It is conceivable that directing against TGF-β is too narrow an approach in humans. Other factors besides TGF-β are also involved, as are other factors that may mediate the effects of TGF and scar formation.

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may have potential clinical applications in reducing postoperative scarring after glaucoma surgery.

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