

Protein Expressions of the Small GTPase Rho Proteins in Pterygial Tissue and Leukocytes of Patients with Pterygium [†]

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Abstract: Pterygium is a benign fibrovascular proliferation that develops from the conjunctiva and invades the cornea. The etiology of this disorder remains unclear. Current treatment of pterygium is surgical. The postoperative recurrence rate of pterygium is reported to be high. To the best of our knowledge, these results are the first to demonstrate the contribution of protein expressions of the small GTPase Rho proteins in patients with pterygium. Our data showed that leukocyte RhoA, RhoB, RhoD, and RhoE protein expressions were markedly elevated in primary pterygium. However, no significant modifications were noted in pterygial tissues.

Keywords: expression; leukocyte; pterygium; Rho proteins

1. Introduction

Pterygium is a proliferative wing-shaped fibrovascular tissue overgrowth arising from bulbar conjunctiva and growing onto the cornea. Pterygium is associated with chronic UV exposure and is characterized by proliferation, inflammatory infiltrates, fibrosis, angiogenesis and extracellular matrix breakdown. The pathogenesis of pterygium formation is not completely understood. Genetic factors are thought to have a role in the development of pterygium. However, the most of the molecular mechanisms leading to pterygium are still unknown. Vascular Endothelial Growth Factor (VEGF) protein expression is significantly high in pterygial specimens, and RhoA/Rho kinase signaling is involved in VEGF-induced endothelial cell migration, hyperpermeability and angiogenesis [1–3]. The purpose of this study was to assess the possible contribution of the small GTPase Rho protein expressions in pterygial tissues and leukocytes of patients with pterygium.

2. Experimental Procedure

A total of 30 patients with primary, and 15 patients with recurrent pterygium were included to this study. Primary and recurrent pterygia and uninvolved superior temporal bulbar conjunctiva specimens were obtained from patients who underwent pterygium excision and conjunctiva autograft surgery. This study was approved by the local Ethics Committee. Protein expressions were analyzed with Western blot. Primary and recurrent pterygial, and conjunctival tissue samples were rapidly frozen in liquid nitrogen and stored at -80°C . Frozen primary and recurrent pterygial, and conjunctival tissue samples were homogenized in 20 mM HEPES (H3375, Sigma Chemical Co., St. Louis, MO, USA) buffer (pH 7.5) including 20 mM β -glycerophosphate (G9422, Sigma Chemical Co., St. Louis, MO, USA), 20 mM sodium pyrophosphate (S6422, Sigma Chemical Co., St. Louis, MO, USA), 0.2 mM sodium orthovanadate (450243, Sigma Chemical Co., St. Louis, MO, USA), 2 mM ethylenediaminetetraacetic acid (EDTA) (E6758, Sigma Chemical Co., St. Louis, MO, USA), 20 mM sodium fluoride (S7920, Sigma Chemical Co., St. Louis, MO, USA), 10 mM benzamidine (434760, Sigma Chemical Co., St. Louis, MO, USA), 1 mM dithiothreitol (DTT) (43815, Sigma Chemical Co., St. Louis, MO, USA), 20 mM leupeptin (L5793, Sigma Chemical Co., St. Louis, MO, USA), and 10 mM aprotinin (A1153, Sigma Chemical Co., St. Louis, MO, USA) using a homogenizer (TissueLyser LT, Qiagen, Hilden, Germany), and kept at -80°C until Western blot analysis. Lysis buffer (79217, Erythrocyte Lysis Buffer, Qiagen, Hilden, Germany) was used for blood samples, and lysate was used for protein and Western blot assay. Bicinchoninic acid protein assay kit (cat. no.23227, Thermo Fisher Scientific, Rockford, IL, USA) was used to determine protein concentration. All samples were electrophoresed in 10% sodium dodecyl sulfate (SDS) (L3771, Sigma Chemical Co., St. Louis, MO, USA)-polyacrylamide gels (A3574, Sigma Chemical Co., St. Louis, MO, USA). The proteins were then transferred to polyvinylidenedifluoride (PVDF) (#1620177, Bio-Rad, Hercules, CA, USA) membranes. The blots were blocked for 1 h in 5% nonfat milk (#1706404, Bio-Rad, Hercules, California, USA) in Tris buffered saline (TBS, pH 7.4) and 0.1% Tween-20 (P9416, Sigma Chemical Co., St. Louis, MO, USA) (TBST). PVDF membranes were incubated overnight with primary antibodies and β -actin antibody was used as a loading control. The blots were incubated overnight at 4°C with primary antibody against RhoA (sc-418, 1:500, Santa Cruz Biotechnology, Dallas, TX, USA), RhoB (sc-8048, 1:500, Santa Cruz Biotechnology, Dallas, TX, USA), RhoC (sc-393090, 1:500, Santa Cruz Biotechnology, Dallas, TX, USA), RhoD (sc-376340, 1:500, Santa Cruz Biotechnology, Dallas, TX, USA), RhoE (ab171799, 1:1000, Abcam, Cambridge, UK), and a rabbit polyclonal antibody against β -actin (ab16039, 1:1000; anti-beta actin antibody loading control, Abcam, Cambridge, UK). After incubation with horseradish peroxidase secondary anti-mouse IgG (sc-516102, 1:1000, Santa Cruz Biotechnology, Dallas, TX, USA) at room temperature for 1 h and horseradish peroxidase coupled goat polyclonal secondary antibody to mouse IgG-HRP (ab6728, 1:3000, Abcam, Cambridge, UK) at room temperature for 1 h and horseradish peroxidase coupled goat polyclonal secondary antibody to rabbit IgG-HRP (ab6721, 1:3000, Abcam, Cambridge, UK) at room temperature for 1 h, immunoreactivities of the bands were visualized with chemiluminescence substrate Super Signal West Pico (cat. no. 34080, Thermo Fisher Scientific, Rockford, Illinois, USA). The density of each band was measured using an automatic electrophoresis gel image analysis system (Image Lab, Bio-Rad, Hercules, CA, USA), then normalized to beta-actin levels. ANOVA was used for statistical comparisons and a p value of <0.05 was considered statistically significant [4,5].

3. Results

The findings of the present study demonstrated that leukocyte RhoA ($p < 0.01$), RhoB ($p < 0.001$), RhoD ($p < 0.05$), and RhoE ($p < 0.05$), but not RhoC, protein expressions were markedly elevated in primary pterygium. There were no marked changes in leukocyte Rho protein expressions in patient with recurrent pterygium. We studied five Rho protein expressions in primary and recurrent pterygial tissues, but no significant modifications were observed ($p > 0.05$).

4. Discussion

This is the first study to examine the role the small GTPase Rho protein expressions in pterygial tissues and leukocytes of patients with pterygium. Our results suggest that peripheral leukocytes Rho protein expressions may be important, and contribute to the pathophysiology of the pterygium formation.

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Conflicts of Interest: The authors declare no conflict of interest.

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