·Letter to the Editor·

Overexpression of carbonic anhydrase 1 in pterygium

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Dear Sir,

Carbonic anhydrase (CA) is a kind of zinc enzyme that reversibly catalyzes hydration of CO_2. Sixteen CA isoenzymes have been described in mammals [1]. These isozymes play physiological roles in erythrocytes, including CO_2 transport, ion secretion, pH regulation and so forth. CA1 and 2 are both cytosolic enzymes that are found in large quantities in erythrocytes. Excluding hemoglobin, CA1 is the most abundant protein in erythrocytes [2]. CA2 and CA4 are abundant in the non-pigmented ciliary epithelium in adults and this provides the basis of using CA inhibitors to reduce the rate of aqueous humour secretion in order to lower intraocular pressure in glaucoma patients [3]. CA9 is highly expressed in the anterior fibrous tissues connecting with the palpebral conjunctiva [4]. Pterygium is a common disease of the ocular surface that is originally considered as a condition closely associated with exposure of the eyes to excessive sun light [5]. The characteristics of pterygium are proliferation, inflammatory infiltrates, fibrosis, angiogenesis and extracellular matrix breakdown [6], which will result in astigmatism, visual axis occlusion or tear film disturbance. So far there has been no investigation elaborating the expression of CA1 in pterygium patients. In this study, we revealed that CA1 expression in pterygium is higher than that in normal conjunctiva.

We obtained 10 human pterygium specimens and 5 normal conjunctiva tissues for this study. Parts of 5 human conjunctiva and 5 pterygium tissues were treated as we reported before to extract protein by homogenization and centrifugation (12000× rpm, 4℃ for 15min) for SDS-polyacrymide gel electrophoresis (SDS-PAGE), Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry

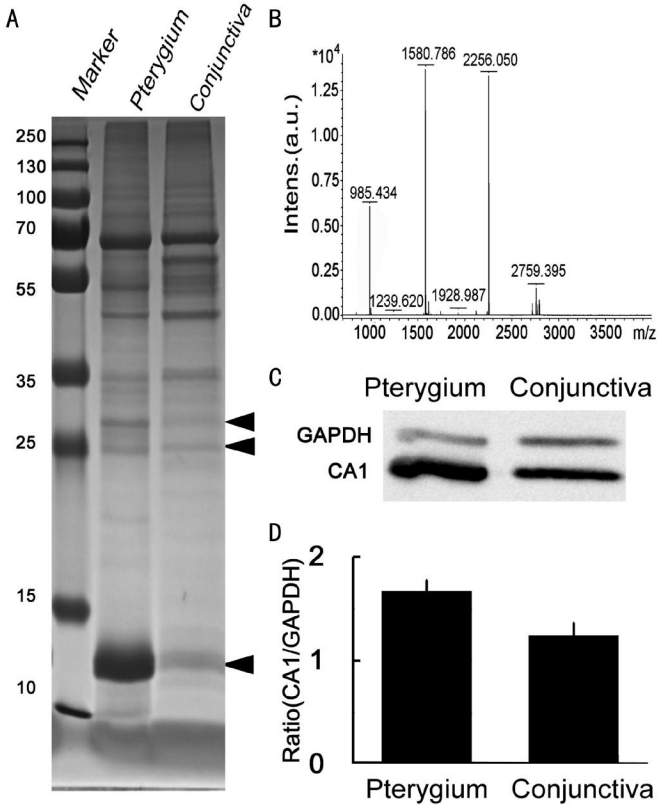

Figure 1 Identification of CA1 in pterygium and normal conjunctiva A: SDS-PAGE pattern of two tissues; B: Peptide mass fingerprinting of CA1; C: Western blotting assay for CA1; D: Quantitative comparison of CA1 in C.

(MALDI-TOF-MS) and Western blot analyses [7-8]. The remaining parts of the specimens were used to do haematoxylin and eosin (H&E) staining and immunohistochemistry assay with being processed for paraffin embedding. The MALDI-TOF/TOF mass spectrum was used to analyze three bands of the SDS-PAGE and identified as CA1, serum amyloid and hemoglobin (Figure 1A, arrow heads, up to bottom). Figure 1B showed that three parent ions (*e.g.* 985.434, 1580.786 and 2256.050) were chosen for the MS/MS spectrum analysis to identify CA1. From the results of SDS-PAGE and Western blot (Figure 1A, 1C, 1D), we could see that CA1 expression in pterygium is higher than that in normal conjunctiva (P<0.01, t-test, n=5). In addition, CA1 expression in pterygium and normal conjunctiva was investigated morphologically (Figure 2). By H&E staining (Figure 2A, 2B), much more fibroplasia and neovascularization could be observed in pterygium compared with conjunctiva. Immunohistochemical staining revealed that CA1 was more intensively expressed in human

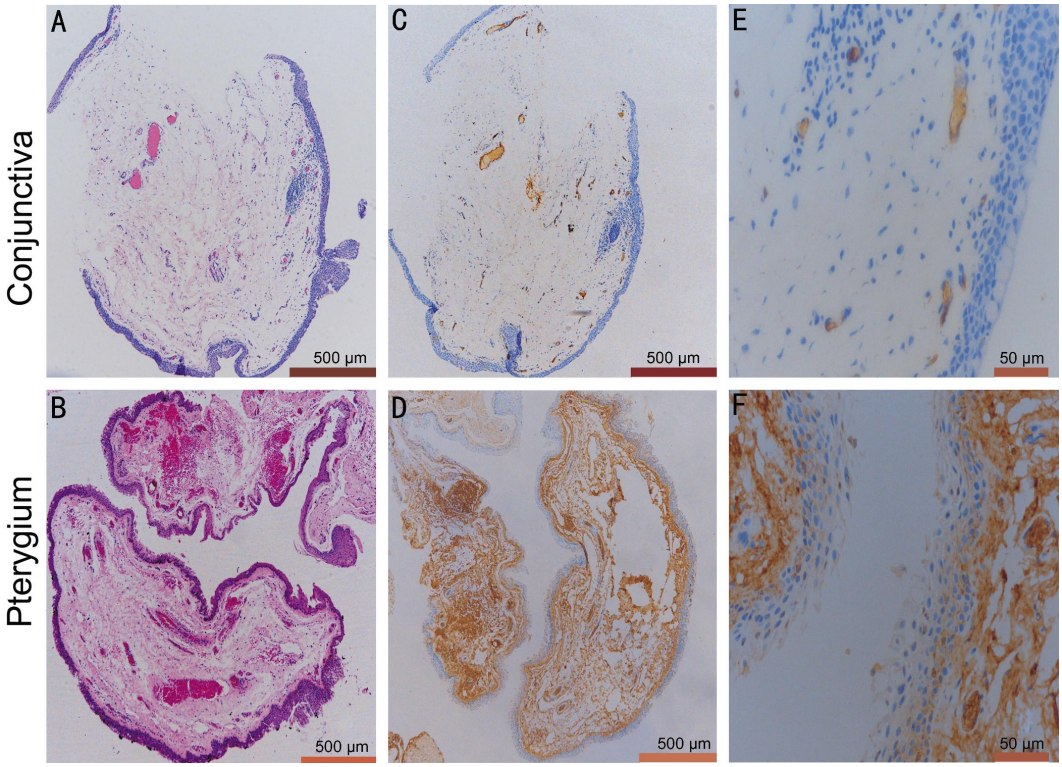

Figure 2 Immunohistochemistry analysis of CA1 expression A, B: H&E staining; C-F: CA1 expression in normal conjunctiva tissue (C, E) and in pterygium (D, F). Scales are 500 μm (A, B, C, D) and 50 μm (E, F).

pterygium tissue (Figure 2D, 2F) than in normal conjunctiva tissue (Figure 2C, 2E). Both pterygium and conjunctiva sections showed strong immunoreactivity for CA1 in erythrocytes. Additionally, epithelial cells of pterygium displayed strong immunoreactivity for CA1, while epithelial cells in normal conjunctiva did not show positive staining. No positive immunoreactivity developed in either the conjunctiva or the pterygium when tissue sections were incubated without anti-CA1 antibody (result not shown). The results indicated that the high levels of CA1 in pterygium is due to the neovascularization and congestion of red blood cells as well as its overexpression in epithelial cells and the subepithelial tissue. Since the primary function of CA1 is to interconvert carbon dioxide and bicarbonate to maintain acid-base balance in blood and other tissues [2], we suggest that CA1 may play a role in pterygium formation. The present result therefore could serve as the basis of future studies of the causal relationship between CA1 and pterygium, including using CA1 as a target for the therapy of primary or recurrent pterygium. In future, much more studies are required to explore the role of CA1 overexpression in pterygium.

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REFERENCES

1 Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nat Rev Drug Discov* 2008;7(2):168-181.

2 Sly WS, Hu PY. Human carbonic anhydrases and carbonic anhydrase deficiencies. *Annu Rev Biochem* 1995;64:375-401.

3 Shahidullah M, To CH, Pelis RM, Delamere NA. Studies on bicarbonate transporters and carbonic anhydrase in porcine nonpigmented ciliary epithelium. *Invest Ophthalmol Vis Sci* 2009;50(4):1791-1800.

4 Abe S, Nakao T, Yoshimoto T, Parkkila S, Murakami G, Cho BH. Expression of carbonic anhydrase in the fetal eye and extra-ocular tissues. *Okajimas Folia Anat Jpn* 2013;90(3):59-68.

5 Nolan TM, DiGirolamo N, Sachdev NH, Hampartzoumian T, Coroneo MT, Wakefield D. The role of ultraviolet irradiation and heparin-binding epidermal growth factor-like growth factor in the pathogenesis of pterygium. *Am J Pathol* 2003;162(2):567-574.

6 Solomon A, Li DQ, Lee SB, Tseng SC. Regulation of collagenase, stromelysin, and urokinase-type plasminogen activator in primary pterygium body fibroblasts by inflammatory cytokines. *Invest Ophthalmol Vis Sci* 2000;41(8):2154-2163.

7 Cui D, Pan Z, Zhang S, Zheng J, Huang Q, Wu K. Downregulation of c-Myc in pterygium and cultured pterygial cells. *Clin Experiment Ophthalmol* 2011;39(8):784-792.

8 Li K, Liu X, Chen Z, Huang Q, Wu K. Quantification of tear proteins and sPLA2-IIa alteration in patients with allergic conjunctivitis. *Mol Vis* 2010; 16:2084-2091.