Supplementary Online Content


eAppendix. Supplemental methods.

This supplementary material has been provided by the authors to give readers additional information about their work.

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eAppendix. Supplemental methods.

**Human conjunctival and corneal epithelial cells**

Our study was approved by the institutional review board of Kyoto Prefectural University of Medicine, Kyoto, Japan; all experiments were conducted in accordance with the tenets of the Declaration of Helsinki. For ELISA and quantitative RT-PCR, we used primary human conjunctival epithelial cells (PHCjE) and immortalized human corneal-limbal epithelial cells (HCLE). PHCjE were harvested from conjunctival tissue obtained at conjunctivochalasis surgery and cultured as follows. Conjunctival tissues were washed and immersed for 1 hr at 37°C in 1.2 U ml⁻¹ purified dispase (Roche Diagnostic Ltd., Basel, Switzerland), and epithelial cells were detached, collected, and cultured in low-calcium defined keratinocytes-SFM medium (dk-SFM; Invitrogen, Carlsbad, CA) with defined growth-promoting additives including insulin, epidermal growth factor, fibroblast growth factor, and 1% antibiotic-antimycotic solution. Using this method, cell colonies usually became obvious within 3 to 4 days. Cultured PHCjE were used after reaching 80% confluence in 7 to 10 days. HCLE, a gift from Dr. Irene K. Gipson, were also cultured in low-calcium dk-SFM medium with defined growth promoting additives including insulin, epidermal growth factor and fibroblast growth factor, and 1% antibiotic-antimycotic solution and used after reaching 80% confluence.

**Quantitative reverse transcription polymerase chain reaction (RT-PCR) assay**

Total RNA was isolated from PHCjE and HCLE using the RNeasy mini kit (QIAGEN, Valencia, CA) according to the manufacturer’s instructions. For the RT reaction we used the SuperScript™ preamplification kit (Invitrogen). Quantitative RT-PCR was on an ABI-prism 7700 instrument (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions. The primers and probes for human MCP-1 (CCL2) were from Applied Biosystems. The results were analyzed with sequence detection software (Applied Biosystems). Quantification data were normalized to the expression of the housekeeping gene GAPDH.

**ELISA**

We performed ELISA to confirm protein production. The amount of MCP-1 released into the culture supernatant was determined by ELISA using the the OptEIA™ human MCP-1 set (BD Pharmingen, San Diego, CA) in accordance with the manufacturer’s instructions.

**Data Analysis**

Data were expressed as the mean ± SE and evaluated by Student’s t-test using the Excel program.

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