Expression of beta defensin genes in frozen thawed and cultured immortalised human corneal epithelial cell line

ABSTRACT

Human beta defensins (hBD) are important host defense molecules at the ocular surface. In addition to their antimicrobial activities, hBD may also act as regulatory factors in recruiting and activating immune cells. Only hBD-1 – hBD-4 have been well characterised. To date, a complete profile of the beta defensin genes (DEFB) expression in immortalised human corneal epithelial cell line (HCE-2) has not been established. Therefore, this study is aimed to explore a spectrum of DEFB expression in HCE-2. Total RNAs were extracted from frozen thawed HCE-2 and cultured HCE-2 to ensure the gene expression were identical. The RNAs were reverse transcribed into cDNAs. The expression of 10 DEFB (DEFB1, DEFB4A, DEFB103, DEFB104, DEFB105, DEFB106, DEFB109, DEFB123, DEFB126 and DEFB127) were analysed using polymerase chain reaction (PCR) and gel electrophoresis. DEFB1 and DEFB103 were the only hBD mRNAs found constitutively expressed in frozen thawed HCE-2 and cultured HCE-2. It was also interesting to note that PCR enhancer was needed to amplify the genes in cultured HCE-2. Our findings suggest that corneal epithelium constantly produce hBD-1 and hBD-3, which presumably provide the baseline defense against infection. Further investigation on the expression of these genes when HCE-2 stimulated with proinflammatory cytokines would help in better understanding of the ocular surface defense mechanism.

Keyword: Beta defensins; DEFB; Human corneal epithelium; HCE-2; Frozen thawed