CHARACTERIZATION OF MORPHOLOGY AND GROWTH RATE OF STABLY TRANSFECTED MDCK CELL LINE, EXPRESSING WILD TYPE OF HBEST1 PROTEIN

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ABSTRACT

Bestrophins are transmembrane proteins found in many organisms. Human bestrophin-1 (hBest1) is expressed in astrocytes and in the basolateral membrane of the retinal pigment epithelium cells (RPE). Malfunctions of the protein lead to retinal pathologies, referred as bestrophinophaties. Bestrophin-1 is thought to be a calcium-dependent chloride channels. Also, there are evidences that Best1 conducts thiocyanate, glutamate and bromine ions and γ-aminobutiric acid. For a better understanding of protein function we created a MDCK cell line stably expressing hBest1 protein. To determine the influence of the new protein on cell's vitality, we analyzed the growth rate, mitotic index and morphology of stably transfected and non-transfected cells. Our observations indicated no difference in these characteristics in transfected and non transfected cells.

***Key words**: Best1, MDCK*

Introduction

hBest1 protein is encoded by *hBEST1* gene, which is located in chromosome 11. Mutations in *hBEST1* have been linked to several forms of retinopathies including Best vitelliform macular dystrophy (Best disease), adult onset macular dystrophy, autosomal dominant vitreoretinochoroidophaty, autosomal recessive bestrophinopathy [1, 8]. It has been suggested that Best1 mutations are also responsible for a subset of retinitis pigmentosa [3]. Best disease is autosomal-dominant, progressive, juvenile-onset macular degeneration associated with large deposits of a yellow pigmented material in the subretinal and sub-RPE spaces. It leads to progressive loss of the central vision [12].

Best1 is transmembrane protein, localized in astrocytes [11] and at basolaterial side of retinal pigment epithelium [6]. Its structure includes four transmembrane domains and the two ends of the protein are intracellular. Best1 is thought to be an ion channel [2, 5]: it was shown to be permeable for chloride, thiocyanate, bicarbonate, glutamate and GABA [4, 9, 10].

To study the protein functions, we established a model system of MDCK II cell line stably transfected with hBest1. Nontransfected MDCK cells do not express endogenously Best1 protein. The presence of stably expressed Best1 protein in transfected MDCK was demonstrated by immunofluoresce studies and Western blot (data not shown). Our studies suggest that Best1 protein doesn't influence the cells' morphology, growth ratio and mitotic index of transfected cells.

Materials and methods

MDCK II cells were grown in DMEM (Sigma) with 1% Penicillin/Streptomycin (Sigma) and 10% fetal bovine serum (Sigma) at 37°C and 5% CO_2. The transfection was performed using a

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vector p.Receiver containing the human *BEST1* gene, (imaGenes GmbH, Berlin, Germany), by Effectene® transfection reagent (Qiagen, Bulgaria) according to manufacturer's instructions [7]. The transfected cells were selected with 500 mg/ml G418 for 14 days.

Two concentrations (1.10^4 and 5.10^4 cells/ml) of transfected and nontransfected MDCK cells for growth curves were used. The number of cells was calculated every 24 hours in a period of seven days. For mitotic index initial cell concentration of 5.10^4 cells/ml was used. Cells were grown on cover slips and fixed for 15-20 min with 70% ethanol on every 24 hours for 6 days. After fixation, cells were stained with Giemsa. On randomly chosen areas mitotic and nonmitotic cells were counted. Mitotic index was calculated as a percentage of mitotic cells from the total cell population. On the same cover slips the cells morphology was examined.

Results and discussion

The two cell lines showed no differences in their growth characteristics (Fig. 1 and 2). On the growth curves both cell lines transited from one phase of growth to the following at the same time. Cells seeded at an initial concentration of 1.10^4 cells/ml do not reach the stationary phase for the duration of the experiment. Instead, cells with concentration 5.10^4 cells/ml reach the stationary phase on day 6th.

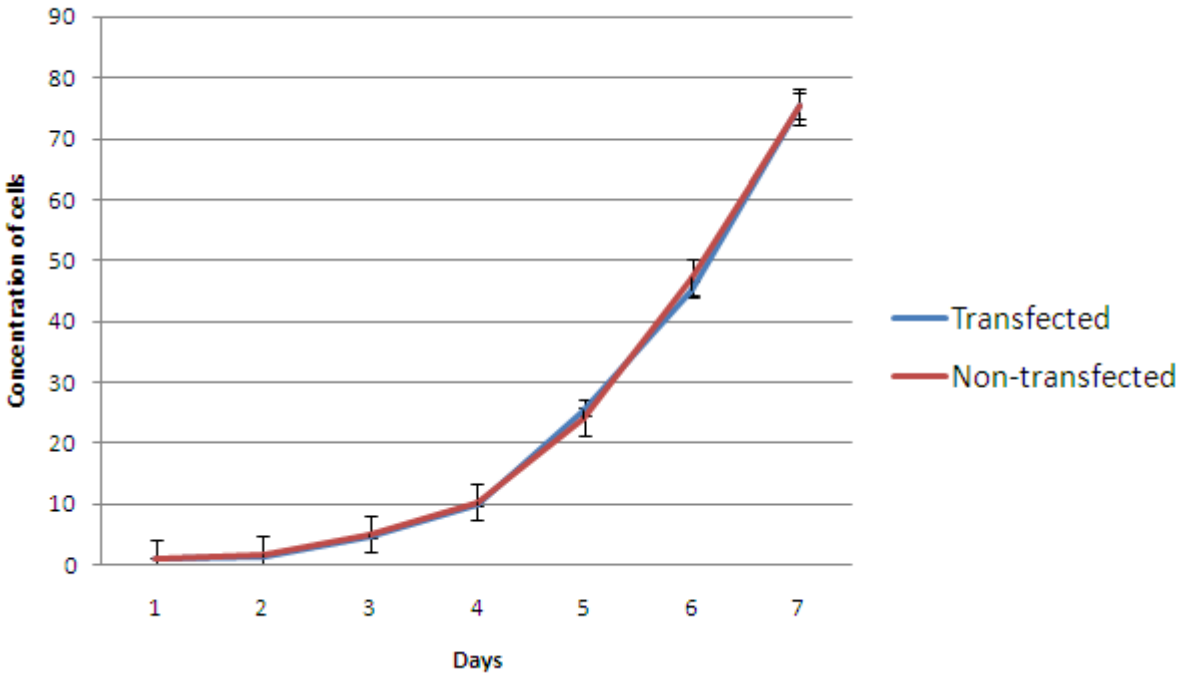


Fig.1 Growth curves of transfected and non-transfected MDCK cell lines at initial concentrations of 1.10^4 cells per ml.

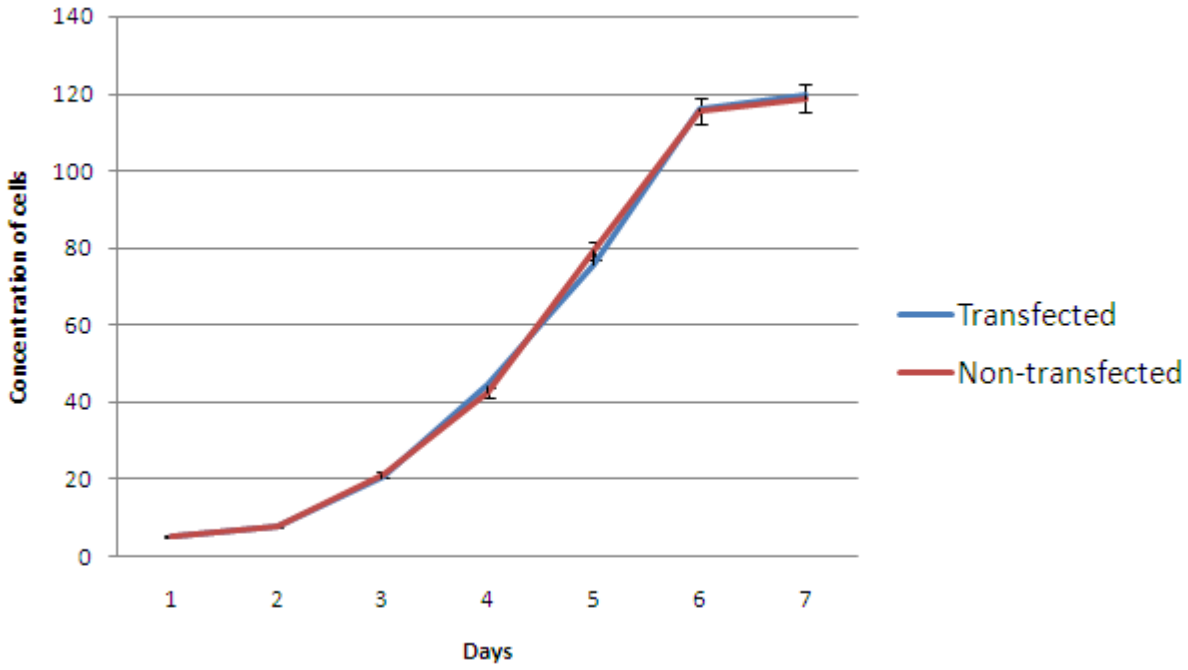


Fig.2 Growth curves of transfected and non-transfected MDCK cell lines at initial concentrations of 5.10^4 cells per ml.

The mitotic index of transfected cells didn't differ from those of non-transfected cells (Fig.3). The mitotic index of transfested cells was systematically higher than non-transfected, but the differences were not statistically reliable. Examination of permanent microscope slides revealed no morphological differences between these two cell lines (Fig.4).

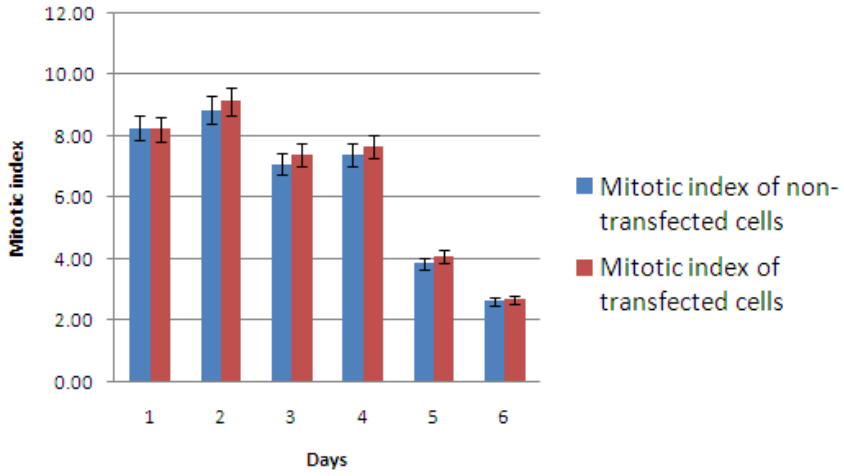
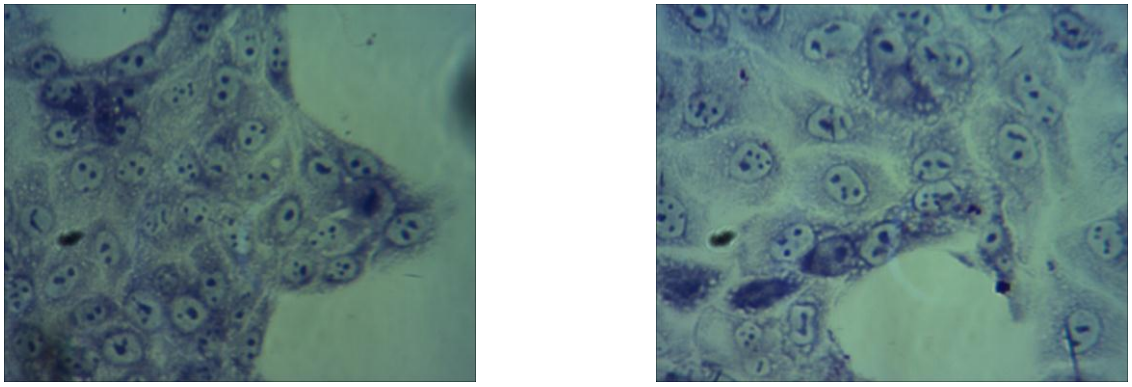


Fig.3 Mitotic index of transfected and non-transfected MDCK cell lines at initial concentrations of 5.10^4 cells per ml.



a) b)

Fig.4 Morphology of non-transfected (a) and transfected (b) MDCK II cells. Giemsa staining, 400 x.

Our observations revealed no differences in morphology, growth rate and mitotic index between transfected with hBest1 and non-transfected MDCK II cells. We consider that stably transfected MDCK cell line, expressing hBest1, can be used as a proper model system for investigations of the structure and functions of hBest1.

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