INVESTIGATION OF CELLULAR LOCALIZATION OF BESTROPHIN-1 MUTANT Y227N IN RETINAL EPITHELIAL CELLS


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ABSTRACT:
Bestrophin-1 (Best1) is a specific for the retinal pigment epithelium (RPE) protein. It is an integral transmembrane protein, localized at the basolateral plasma membrane. It plays a role in the transport of Cl$^-$ and HCO$_3^-$ ions, thus probably regulating cellular volume and pH. Mutations in the protein are connected with number of retinal diseases, called bestrophinopathies. It is shown, that in the histological retinal sections from patient with Best Vitelliform macular degeneration, carrying Y227N mutation, mutant protein is localized not only on the basolateral, but also on the apical side of the RPE cells. We investigated cellular localization of the same mutant protein in the cell line, originated from RPE. Our observation indicated that normal and mutant proteins are expressed in the cells, but we were not able to detect clear polarized localization or retention of the protein in the Golgi apparatus.

Keywords: Best1, RPE-J

Introduction:
Identification of human protein bestrophin-1 (Best1) is a result of identification of gene, responsible for Best Vitelliform macular degeneration (BVMD) in 1998 [5, 10]. This gene is predominantly expressed in the retinal pigment epithelium (RPE), while some expression is noted in the kidney, brain and spinal chord. But protein expression is detected only in RPE cells. Best1 protein is 68kDa integral membrane protein [4, 6, 13, 14], localized at the basolateral plasma membrane of RPE [4]. It functions as an ion channel across the membrane, thus regulating homeostasis of photoreceptors and whole retina [2, 3, 11, 12]. In 2005 Mullins et al. showed that on the histological sections from retina of 93 years old patient, caring Y227N mutation in Best1, who developed BVMD late in his life, protein is partially mislocalized [8] with expression not only on basolateral membrane, but on apical membrane too.

In recent study we investigated cellular localization of Best1 protein, caring the same Y227N mutation into cell line, originated from RPE. Our results confirmed nonpolarized expression of this mutant in cultured retinal epithelium cell line.

Materials and methods:
All reagents and chemicals were supplied by Sigma-Aldrich (St. Quentin Fallavier, France) unless otherwise stated. Antibodies were obtained as follows: mouse IgG1 antibody (E6-6) directed against the C-terminal domain of human Best1 from Novus Biologicals Inc. (Littleton, France) [3, 7, 12], rabbit anti-ZO-1 from Zymed (Invitrogen), rabbit anti-GM-130 from AbCam, rabbit anti-ZO-1 from Zymed (Invitrogen), goat anti-mouse AlexaFluor 488, and goat anti-rabbit AlexaFluor 594 from Invitrogen.
RPE-J cells [9] were maintained in DMEM with 4% FCS, 1% non-essential amino acids, 1% HEPES, streptomycin (100 mg/l) and penicillin (60 mg/l) at 32°C and 5% CO₂. Transfections of cells were performed 24 hours after cell seeding using the Effectene® Transfection Reagent (Qiagen). Transiently transfected cells expressing human Best1 or Y227N mutant were grown for five days on coverslips and fixed with ice-cold methanol. After blocking with PBS++ containing 1% BSA for one hour, cells were incubated with a mouse anti-Best1 and rabbit ZO-1 overnight at 4°C. Bound antibodies were detected using proper secondary antibodies for two hours at room temperature. For negative controls, samples were incubated with the secondary antibody alone. Nuclei were labeled with DAPI. Best1 fluorescence was visualized using Leica TCS SP5 confocal microscope and LAS AF software (Leica Microsystems), with a HCX PL APO lambda blue 63.0x1.40 OIL objective and 4-time zoom enlargement. Z- and Y-series image data were acquired in 0.35- and 0.30-µm step size sequential scanning respectively.

**Results and discussion:**

We investigated cellular localization of normal and mutant human Best1 in the rat retinal epithelial cell line RPE-J. It is known, that during immortalization, retinal cell lines lose expression of some specific protein, including Best1 [1]. This could make them, after transfection, a model system for investigation of expression and cellular localization of recombinant Best1 proteins.

![RPE-J cells, transiently transfected with normal human Best1 (green). Nuclei are labeled with DAPI (blue) and tight junction marker ZO-1 is in red. Scale bar for x-y planes is 25µm and for x-z planes is 10µm.](image)

As it is shown on Fig.1, five day after transfection some of the cells are positive for Best1 (green). Although cells posses well developed tight junctions, visualized by the ZO-1 protein (red) we were not able to detect polarized localization of Best1 and preferential expression on the basolateral membrane. Beside on the membrane, a big amount of the protein showed intracellular localization, indicating probable retention into the biosynthetic pathway, caused by the over-
expression. Co-localization with Golgi apparatus marker GM-130 (Fig. 2) revealed partial overlapping of the signals. But the major amount of Best1 signal did not co-localized with Golgi apparatus.

Fig. 2 RPE-J cells, transiently transfected with normal human Best1 (green). Co-localization with trans-Golgi marker GM130 (red). Nuclei are labeled with DAPI (blue). Scale bar for x-y planes is 25µm and for x-z planes is 10µm.

Fig. 3 RPE-J cells, transiently transfected with Best1 mutant Y227N (green). Nuclei are labeled with DAPI (blue) and tight junction marker ZO-1 is in red. Scale bar for x-y planes is 25µm and for x-z planes is 10µm.
Tyrosine at the position 227 into the Best1 is a part of presumable sorting motive Y227DWI. Disruption of this motive could affect proper cellular localization of the protein.

Our observations revealed that after transfection Y227N mutant is synthesized into the RPE-J cells. The protein is localized on the cell surface and inside the cells with some perinuclear clustering. Membrane localization was on basolateral but also on the apical membrane. x-z sections revealed that in number of cells Best1 Y227N is with apical, and in other cells is with non-polar (apical and basolateral) localization (Fig. 3).

Perinuclear clustering of Best1 Y227N could be due to the retention of the protein, caused by the mutation. But co-localization with trans-Golgi marker GM-130 indicated that only a part of the Best1 signal co-localize with Golgi apparatus (Fig. 4).

As a transmembrane protein, after synthesis at the Endoplasmic reticulum Best1 is transported to and processed in the Golgi. We considered that Golgi localization of some normal and mutant protein molecules is not caused by retention and accumulation, but these are molecules undergoing processing. As it is seen form confocal images, only a small part of the Best1 signal co-localized with GM-130, supporting this conclusion.

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