Association of bestrophin-1 with membrane domains in model monolayers and epithelial cells - a prerequisite for innovative therapies for retinal degenerations
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Summary:
Molecular interactions in the cell membranes are essential for transmission of matter, energy and information, maintaining the integrity of the cells and cell cycle. The knowledge of structure, organization and topology of membrane proteins and lipids are a prerequisite for establishing the dynamics of intercellular interactions, specificity and mechanisms of signaling pathways in cells. Human bestrophin-1 (hBest1) is a transmembrane protein, predominantly expressed in the membranes of retinal pigment epithelium (RPE) and neuronal cells, a member of Ca2+-dependent anion channel family with still unclear function. Mutations in the BEST1 gene, coding protein hBest1 in the retinal pigment epithelium, are responsible for generalized damages of the retina, known as bestrophinopathies, one of which is Best disease (BVMD – Best Viteliform Macular Degeneration) - the second most common and yet incurable form of juvenile inherited macular degeneration. New biological roles and large social effects of hBest1 have been proposed in 2017, when its participation in the pathology of Alzheimer's, Parkinson's and other brain diseases (stroke, epilepsy, etc.) have been determined. So far there are not sufficient data about: the functional organization and structure of hBest1; its conformational dynamics and orientation; the mechanisms of its interaction, sorting and specific binding to lipid domains in epithelial cell membranes; its participation in cell polarization; its effects on proliferation and differentiation of cells; hBest1 interactions with other

intra- and extracellular proteins as well as pharmacologically active agents, triggering different signaling pathways and their regulation; molecular mechanisms of pathological conditions, etc., which determines interest, importance and actuality of fundamental research with hBest1 ion channel worldwide. Since, hBest1 is expressed primarily in the retinal pigment epithelium, these studies became possible only after application of molecular biological approach and original methodology for producing and purifying functionally active human recombinant hBest1 in stably transfected eukaryotic cells, introduced for the first time by our scientific team. The main objective of the project is to study the interactions of hBest1 and its mutant forms leading to BVMD with membrane domains (raft and non-raft lipids and proteins) and pharmacologically active agents in cell cultures as well in model membrane systems which provides the necessary fundamental knowledge and the ability to apply innovative therapies by using nanostructures such as bilayer polymer-lipid disks, liposomes and bicontinuous structures or polymeric nanosized particles to intercalate the protein in the cell membrane and restore its transport functions. Interdisciplinary approach in this research project requires and will apply Biochemical, molecular and cellular biological experiments, to examine: the mechanism of sorting and binding of hBest1 and its mutant forms to lipid rafts in eukaryotic cells; its participation in cellular polarization, proliferation and differentiation of epithelial cells (in the presence/absence of various ions, biologically and pharmacologically active agents); sorting signals of hBest1, responsible for the proper transport in polarized cells and changes in the cellular localization of various hBest1 mutants; and the role of mutant forms for the pathogenesis of Best disease. Experiments with model membrane systems (monolayer and bilayer model membranes in combination with physical and physicochemical methods - BAM, AFM, PM-IRRAS, etc.) are required for: quantitative investigations and characterization of the specific interactions of hBest1 (and its mutant forms) with raft and non-raft lipids and proteins; and conformational states and functional dynamics of hBest1 in membranes at different lipid composition, temperature and lipid phase transition states, in presence of ions, biologically and pharmacologically active agents. The original results and the successful realization of the proposed research project will provide an up-to-date model for the molecular mechanism of hBest1 activity that will open up opportunities for both the study Best disease pathogenesis and the use of nanotechnologies to improve the quality of life of the affected individuals.

Key words: hBest1, Cellular polarization, Lipid rafts, nanodiscs, liposomes, monolayer and bilayer model membranes