The summaries of the peer-reviewed publications of Dr. Kirilka Mladenova, participating in a competition for "associate professor" announced in the State Gazette no. 30/15/04/2022

1. K. Mladenova, V. MoskovaDoumanova, I. Tabashka, S. Petrova, Z. Lalchev and J. Doumanov **Establishment and haracterization of stably transfected MDCK cell line, expressing hBest1 protein** Bulgarian Journal of Agricultural Science, 2013, 19 (2), 159–162

Summary

Bestrophin-1(Best1) is a transmembrane protein specifically expressed in the retinal pigment epithelial cells. It is localized on their basolateral plasma membrane. Malfunctions of the protein lead to retinal pathologies, named Bestrophinophaties. Best1 is thought to be a Ca2+- activated Cl– channel or a regulator of ion transport, or both. Some other functions in the maintaining of cellular homeostasis are also attributed to this protein. In order to obtain additional information about protein structure and functions we established a stably transfected MDCK II cell line expressing human Best1, conjugated with Myc and His- tags at C-terminus. The presence of stably expressed Best1 protein was demonstrated by immunofluoresce studies. Confocal microscopy images confirmed its basolateral localization. Additionally, no differences in the cell growth ratio and mitotic index of established cell line, in comparison with conventional MDCK cells were observed. Our studies suggest that Best1 does not infl uence cell growth and cell polarity in transfected cells, making our stably transfected cell line an appropriate model for investigations of Best1 functions.

2. Veselina Moskova Doumanova, **Kirilka Mladenova**, Svetla Petrova, Tanya Topouzova-Hristova, Christina Chakarova, Zdravko Lalchev, Jordan Doumanov **Aminoacid exchange R25W affects proper cellular localization of Best1 protein in MDCKII cells**, Comptes rendus de l'Acade'mie bulgare des Sciences, 2014, Tome 67, Number 2

Summary

Bestrophinopathies are rare diseases, linked to the mutations in *BEST1* gene, coding Best1 protein. This is a channel protein, predominantly expressed on the basolateral membrane of the retinal pigment epithelial cells in the eye. It was recently shown that some mutations alter proper cellular localisation of the protein. In a recent study we investigated localisation of Best1 protein, carrying R25W disease causing mutation, in polarised MDCK II cells. Our observations revealed a growing of apical localisation of the protein but not a complete reverse of polarity. Arginine (R) at position 25 is very conservative and lies between two potential sorting motives. It influences proper localization of the protein, even though it is not a potential sorting motif itself.

3. Jordan Doumanov, **Kirilka Mladenova**, Radoslav Aleksandrov, Georgi Danovski, Svetla Petrova, 2014 **Interactions of pharmacologically active snake venom sPLA2 with different cell lines**, Biotechnology and biotechnological equipment, том:28, брой:5, 2014, стр.918-922, ISSN (print):1310-2818, ISSN (online):1314-3530

Summary

Secreted Phospholipases A2 (sPLA2s) represent a large family of structurally related enzymes, which target different tissues and organs and induce numerous pharmacological effects based on their

catalytic specificity – hydrolysis of the sn-2 ester bond of glycerophospholipids. The neurotoxin vipoxin, isolated from the venom of *Vipera ammodytes meriodionalis*, is a heterodimeric postsynaptic ionic complex composed of two protein subunits – a basic and toxic His48 sPLA2 enzyme and an acidic, enzymatically inactive and non-toxic component. In this paper, for the first time, we demonstrate that vipoxin sPLA2 enzyme affects cell integrity and viability of four cell types and causes different cell responses. The most dramatic local tissue effects were observed with RPE-1 (retinal pigment epithelial) cells followed by A549 (adenocarcinomic human alveolar epithelial) cells and MDCK (Madin-Darby Canine Kidney epithelial) cells. Products of the enzymatic reaction, lysophospholipids and unsaturated free fatty acids, act as lipid mediators that can induce membrane damaging or can stimulate cell proliferation. Our preliminary results on the cytotoxic effect of vipoxin sPLA2 on A549 cells are promising in searching of its eventual anticancer potential.

4. Ralitsa Veleva, Bela Petkova, Veselina Moskova-Doumanova, Jordan Doumanov, Milena Dimitrova, Petya Koleva, **Kirilka Mladenova**, Svetla Petrova, Zhenya Yordanova, Veneta Kapchina-Toteva, Tanya Topouzova-Hristova 2015 **Changes in the functional characteristics of tumor and normal cells after treatment with extracts of white dead nettle** Biotechnology and biotechnological equipment, том:29, брой:1, 2015, стр.181-188, ISSN (print):1310-2818, ISSN (online):1314-3530, doi:10.1080/13102818.2014.989094

Summary

Lamium album L. is a perennial herb widely used in folk medicine. It possesses a wide spectrum of therapeutic activities (anti-inflammatory, astringent, antiseptic, antibiotic, antispasmodic, antioxidant and anti-proliferative). Preservation of medicinal plant could be done by in vitro propagation to avoid depletion from their natural habitat. It is important to know whether extracts from L. album plants grown in vitro possess similar properties as extracts from plants grown in vivo. For these reasons, it is important to examine changes in the composition of secondary metabolites during in vitro cultivation of the plant and how they affect the biological activity.

We used A549 human cancer cell line and normal kidney epithelial cells MDCKII (Madin–Darby canine kidney cells II) as controls in assessing the anti-cancer effect of plant extracts. To elucidate changes in some key functional characteristics, adhesion test, MTT (3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyl tetrazolium bromide), transepithelial resistance (TER), immunofluorescence staining and trypan blue exclusion test were performed.

Methanol and chloroform extracts of in vivo and in vitro propagated plants affected differently cancerous and non-cancerous cells. The most pronounced differences were observed in the morphological analysis and in the cell adhesive properties. We also detected suppressed epithelial transmembrane electrical resistance of MDCK II cells, by treatment with plant extracts, compared to non-treated MDCK II cells. A549 cells did not polarize under the same conditions. Altered organization of actin filaments in both cell types were noticed suggesting that extracts from L. album L. change TER and actin filaments, and somehow may block cell mechanisms, leading to the polarization of MDCK II cells

5. T. Topouzova-Hristova, J. Doumanov, D. Melnishka, **K. Mladenova**, V. Moskova-Doumanova, Z. Lalchev, T. Andreeva, E. Haladjova, S. Rangelov 2015 **Effects of novel gene delivery vector systems based on poly(vinyl benzyl trimethylammonium chloride) on A549 cell line** FEBS journal , том:282, брой:S1, 2015, стр.282-282, ISSN (print):1742-464X, ISSN (online):1742-4658, doi:https://doi.org/10.1111/febs.13339

Summary

Polymer-based gene delivery systems are safer, less pathogenic and less immunogenic alternatives to viral systems. In this work we are focused on effects of novel homopolymers based on vinyl benzyl trimethylammonium chloride as gene delivery vector systems in a mode A549 human lung cancerous cell line. Cells are incubated with DNA/polymer complexes (polyplexes contain salmon sperm DNA) at a wide range of N/P (amino-to-phosphate groups) ratios and concentrations for 6 h. Using MTT assays, trypane blue and methylene blue staining, we investigate cytotoxicity of polyplexes, whereas their behaviour into cells during five days period was investigated by microscopy observations. Using MTT assay, we found very low/non toxicity of both the pure polymer and polyplexes at various N/P ratios in the concentration range 5–50 lg/ml of. At the same time, a partial permeabilisation of cellular membranes was detected. Our results suggest absorption on the cell surface and entering of polyplexes into about 50% of the cells. Additionally, 48 h–72 h after treatment, the polyplexes showed movement out of the cells, probably forming exosomes. The cell number dramatically decreased and the cell morphology was affected. Our data suggest successful internalization of the studied polyplexes; however, they stay stable for several days into cytoplasm. For delivery of DNA it is critical to develop less stable polymer particles. We conclude that these nanosized complexes are promise materials to transport biological molecules and particular for gene therapy for treating a wide range of diseases.

6. **Kirilka Mladenova**, Svetla D. Petrova, Tonya D. Andreeva, Veselina Moskova-Doumanova, Tanya Topouzova-Hristova, Yuri Kalvachev, Konstantin Balashev, Shomi S. Bhattacharya, Christina Chakarova, Zdravko Lalchev, Jordan A. Doumanov 2017 **Effects of Ca2+ ions on bestrophin-1 surface films**, Colloids and Surfaces B: Biointerfaces, 2017, 149 (2017) 226–232

Summary

Human bestrophin-1 (hBest1) is a transmembrane calcium-activated chloride channel protein – memberof the bestrophin family of anion channels, predominantly expressed in the membrane of retinal pigment epithelium (RPE) cells. Mutations in the protein cause ocular diseases, named Bestrophinopathies. Here, we present the first Fourier transform infrared (FTIR) study of the secondary structure elements of hBest1, π/A isotherms and hysteresis, Brewster angle microscopy (BAM) and atomic force microscopy (AFM) visualization of the aggregation state of protein molecules dispersed as Langmuir and Langmuir-Blodgettfilms. The secondary structure of hBest1 consists predominantly of β10-helices (27.2%), α-helixes (16.3%), β-turns and loops (32.2%). AFM images of hBest1 suggest approximate lateral dimensions of 100 × 160 Åand 75Å height. Binding of calcium ions (Ca2+) induces conformational changes in the protein secondary structure leading to assembly of protein molecules and changes in molecular and macro-organization ofhBest1 in monolayers. These data provide basic information needed in pursuit of molecular mechanisms underlying retinal and other pathologies linked to this protein.

7. **K. Mladenova**, S. Petrova, T. Andreeva, V. Moskova-Doumanova, Z. Lalchev, J. Doumanov, 2015 **Effect of Ca2+ ions on Bestrophin-1 interaction with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine in surface films**, FEBS journal suppl., 2015, Volume 282, p. 319, Supplement 1

Summary

Bestrophin-1 (Best1) is a transmembrane channel protein, predominantly expressed in the plasma membrane of retinal pigment epithelium (RPE). Best1 is a multifunctional protein that may acts as a Ca2+ -activated chloride channel or/and regulator of voltage-gated Ca2+ channels.

The channel participates not only in the transport of ions (such as Cl-), but also of organic molecules, such as γ-aminobutyric acid (GABA) in glial cells and glutamate in astrocytes and neurons.

Although the structure of Best1 is still uncertain, it is clear that its interactions with the plasma membrane lipids are important for the conformation, oligomerization and functional activity. Therefore, the knowledge of Best1 interactions with biorelevant lipids is of major importance. Such studies have not been performed so far as the protein was not purified to homogeneity. For the first time, our group published an original methodology for isolation and purification of sufficient quantities of functionally active human recombinant Best1 from stably transfected MDCK cells, enabling this research. Our interest has been focused on the effect of Ca2+ ions on Best1 interactions with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) in Langmuir monolayers since it is the most abundant phospholipid of animal cell membranes. The π/A (surface pressure/area) isotherms and compression/expansion isocycles of Best1, POPC and Best1/POPC monolayers were recorded in absence and presence of Ca2+ in the subphase. The effect of Ca2+ on the morphology of monolayers was observed by Brewster angle microscopy (BAM). Our study shows that the incorporation of Ca2+ in the subphase does not change the shape of π/A isotherms but decrease the mean molecular area of Best1, POPC and Best1/POPC monolayers. These results correlate well with BAM images representing that the presence of Ca2+ induces the formation of lipid/protein macromolecular aggregates (Best1/POPC clusters) during monolayers compression. We assume that Ca2+ ions play role for interaction of Best1 with POPC at physiological conditions in the cell.

8. Jordan Doumanov, **Kirilka Mladenova**, Tanya Topouzova-Hristova, Stoyanka Stoitsova, Svetla Petrova , 2015 **Effects of vipoxin and its components on HepG2 cells**, Toxicon, 2015, 94 36-44

Summary

Snake venom Phospholipases A2 (svPLA2) are among the main toxic venom components with a great impact on different tissues and organs based on their catalytic specificity and a variety of pharmacological effects, whose mechanism is still under debate. The main toxic component, isolated from the venom of Vipera ammodytes meridionalis, is the heterodimeric postsynaptic ionic complex vipoxin, composed of a basic and toxic PLA2 enzyme subunit (GIIA secreted PLA2) and an acidic, enzymatically inactive and nontoxic subunit e vipoxin acidic component (VAC). This study demonstrates for the first time that vipoxin and its individual subunits affect integrity and viability of HepG2 cells displaying differences in their pharmacological activities. Under the experimental conditions, the individual PLA2 subunit induces cytotoxicity, cytoskeletal rearrangements and triggers early apoptosis in a concentration-dependent manner related to its enzymatic activity. Vipoxin and VAC do not affect cell viability but manifest high degree of genotoxicity, whereas DNA damage induced by PLA2 subunit could be defined as moderate and not associated with its catalytic activity. Our results suggest that the interactions between vipoxin subunits play an important role in HepG2 cell response and most likely affect the observed distinction between cyto- and genotoxicity.

9. J. Doumanov, **K. Mladenova**, T. Topouzova-Hristova, I. Ivanova, S. D. Petrova2015, **Influence of snake venom Phospholipase A2 on RPE-1 cells – multiple biological roles of sPLA2,** FEBS journal suppl., 2015, Volume 282, p. 223, Supplement 1

Summary

Secreted phospholipases A2 (sPLA2, EC 3.1.1.4) catalyse the hydrolysis of the 2-acyl ester bond of 1,2-diacyl-3-sn-phosphoglycerides in a calcium-dependent manner, releasing functional activity acting as potent lipid mediators involved in membrane damaging, cell proliferation, inflammation and apoptosis. Snake venom sPLA2 represent a family of structurally related enzymes that affect different type of tissues and provoke neurotoxicity, myotoxicity, cardiotoxicity, anticoagulant effects, nephrotoxicity, hepatotoxicity, platelet aggregation, hemolytic activity, inflammation, and etc. Our

interest has been focused on the toxic effects of snake venom sPLA2 on retinal pigment epithelium (RPE). RPE cells play a key role in phagocytosis of photoreceptor outer segment membranes, absorption of light, visual cycle, secretion, epithelial transport (homeostasis), etc. To shed light on the influence of neurotoxic sPLA2 on RPE cell metabolism and proliferation, we investigate the effects of vipoxin sPLA2 subunit on RPE-1 model cell line. Vipoxin is the main neurotoxin in the venom of Vipera ammodytes meriodionalis snake (endemic to some arias of the Balkans). It is a heterodimeric protein composed of a basic and toxic GIIA sPLA2 subunit (Mr 13828 Da, pI 10.4) and an acidic, enzymatically inactive and nontoxic subunit (Mr 13639 Da, pI 4.6) associated spontaneously in a tight 1:1 complex by multiple non-covalent bonds and additionally stabilized by electrostatic interactions. Separation of vipoxin subunits was performed using cationexchange chromatography on Mono S FPLC column and their homogeneity was assessed by SDS PAGE. We use MTT and comet assays to elucidate cyto- and geno- toxicity, and actin fluorescence staining to detect cytoskeleton rearrangements induced by pure toxic sPLA2. Our results suggest changes in cell metabolic activity, dramatic actin cytoskeleton rearrangement in RPE-1 cells and generation of double-strand DNA brakes. We assume that the products of sPLA2 enzymatic activity have their own impact and affect also cell survival pathways.

10. Kostadinova, A., J. Doumanov, D. Moyankova, S. Ivanov, **K. Mladenova**, D. Djilianov, T. Topouzova-Hristova 2016 **Haberlea rhodopensis extracts affect cell periphery of keratinocytes**, Comptes rendus de l'Acad´emie bulgare des Sciences, 2016, Tome 69, Number 4, pages 439- 448

Summary

Common features of chronic dermatological conditions are inflammation and ROS over generation, as well as disturbances in cell proliferation and differentiation. Our aim was to study the impact of Haberlea rhodopensis extracts on the mitochondrial activity, integrity of cell membranes, actin cytoskeleton and tight junctions (the loss of ZO-1 protein) of the human keratinocytes (HaCaT cells). Cytotoxity tests such as MTS, LDH assay and trypan blue exclusion assay were performed to evaluate metabolic activity and membrane permeability of the cells. In concentrations up to 2 mg/ml the extracts influence cell periphery, permeabilize the membrane and disrupt tight junctions of HaCat keratinocytes, which is more pronounced in actively dividing cells (–Ca+ cells). Our results show that extracts of Haberlea rhodopensis could be a good candidate to be used in complex treatment of pathological dermatological conditions.

11. Kalinova, R., Doumanov, J., **Mladenova, K**., Janevska, D.; Georgieva, M., Miloshev, G., Topouzova-Hristova, T., Dimitrov, I. 2017 **Rational design of polypeptide-based block copolymer for nonviral gene delivery**, ChemistrySelect, 2017, 2, 12006 –12013

Summary

The present work describes the development, characterization, and in vitro evaluation of novel poly(L-lysine)-based polyplexes as nonviral gene delivery systems. Initially, a welldefined hybrid block copolymer comprising poly(ethylene glycol) methacrylate) (POEGMA) and poly(L-lysine) (PLL) blocks was successfully synthesized and characterized. The hybrid copolymer shows high ability to condense DNA into stable polyplexes in aqueous media with sizes of approx. 100 nm. The nanoplexes were evaluated for cellular toxicity in A549 alveolar and HepG2 (hepatocarcinoma) cell lines. The nanoparticles cell internalization and transfection ability were assessed in HepG2 cells. The initial experiments showed that DNA was successfully transfected into the nucleus of human liver cancer cells and expressed enhanced green fluorescent protein (EGFP) gene with green fluorescence emission. These results revealed that the newly synthesized POEGMA-b-PLL diblock copolymer might be very attractive candidate as a nonviral gene delivery vector.

12. Haladjova, E.; Halacheva, S.; Momekova, D.; Moskova-Doumanova, V.; Topouzova-Hristova, T.; **Mladenova, K.;** Doumanov, J.; Petrova, M.; Rangelov, S 2017 **Polyplex Particles based on Comb-Like Polyethylenimine/Poly(2-ethyl-2-oxazoline) Copolymers: Relating Biological Performance with Morphology and Structure**, Macrom. Biosci. – submitted October 2017; ISSN 1616-5195, accepted December 2017, Apr;18(4):e1700349, doi: 10.1002/mabi.201700349. Epub 2018, Feb 28

Summary

The present contribution is focused on feasibility of using comb-like copolymers of polyethylenimine with poly(2-ethyl-2-oxazoline) (LPEI-comb-PEtOx) with varying grafting densities and degrees of polymerization of PEI and PEtOx to deliver DNA molecules into cells. The copolymers form small and well-defined particles at elevated temperatures, which are used as platforms for binding and condensing DNA. The electrostatic interactions between particles and DNA result in formation of sub-100 nm polyplex particles of narrow size distribution and different morphology and structure. The investigated gene delivery systems exhibit transfection efficiency dependent on the copolymer chain topology, shape of the polyplex particles, and internalization pathway. Flow cytometry shows enhanced transfection efficiency of the polyplexes with elongated and ellipsoidal morphology. The preliminary biocompatibility study on a panel of human cell lines shows that pure copolymers and polyplexes thereof are practically devoid of cytotoxicity.

13. Tonya D. Andreeva, Svetla D. Petrova, **Kirilka Mladenova**, Veselina Moskova-Doumanova, Tanya Topouzova-Hristova, Yulia Petseva, Nikola Mladenov, Konstantin Balashev, Zdravko Lalchev, Jordan A. Doumanov 2018 **Effects of Ca2+, Glu and GABA on hBest1 and composite hBest1/POPC surface films**, Colloids Surf B Biointerfaces, 2018 Jan 1;161:192-199.

Summary

Bestrophinopathies are ocular diseases caused by mutations in the human bestrophin-1 (hBest1) – trans-membrane Ca2+ -activated chloride channel protein, mainly expressed in the retinal pigment epithelium (RPE) cells. hBest1 is also an important transporter for neurotransmitters such as glutamate (Glu) and γ-aminobutyric acid (GABA) in the nervous system. Recently, a new biological role of hBest1, related to its possible involvement in the pathology of brain diseases (Alzheimer's, Parkinson's disease) has been proposed. Here, we report the effects of Ca2+ , Glu and GABA on hBest1 and composite hBest1/POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, POPC) Langmuir and Langmuir-Blodgett monolayers based on surface dynamics (π/A isotherms, hysteresis and compressibility), morphology (Brewster angle microscopy, BAM) and visualization of protein molecular organization (Atomic force microscopy, AFM). Ca2+ ions and neurotransmitters Glu and GABA affect hBest1 topology at the air/water interface altering its surface activity, size, orientation and organization. In contrast, no significant changes were detected on π/A isotherms and hysteresis of the composite hBest1/POPC films but their effects on structure, aggregation state and orientation hBest1 established by BAM and AFM differentiate. We found that the binary films of hBest1 and POPC are phase separated at the air/water interface, suggesting stronger lipid-lipid and protein-protein interactions than lipid-protein interactions that can significantly alter the molecular organization and activity of hBest1 in cell membranes. Our data shed light on structure, surface behavior and organization of hBest1 that define relationship structurefunctional activity of hBest1 as transport channel.

14. Pavel Bakardzhiev, Natalia Toncheva-Moncheva, **Kirilka Mladenova**, Svetla Petrova, Pavel Videv, Veselina Moskova-Doumanova, Tanya Topouzova-Hristova, Jordan Doumanov and Stanislav Rangelov 2020 **Assembly of Amphiphilic Nucleic Acid–Polymer Conjugates into**

Complex Superaggregates: Preparation, Properties, and in vitro Performance, European Polymer Journal, Volume 131, 15 May 2020, 109692

Summary

Nucleic acid-polymer conjugates (NAPCs) are obtained by click coupling reactions of appropriately functionalized oligonucleotides with synthetic polymer chains of different chemical nature, composition, and properties, namely, polyethers such as poly(ethoxyethyl glycidyl ether) and polyesters such as poly(ε-caprolactone). The resulting NAPCs are amphiphilic and form stable aggregates in aqueous solution. The aggregates are thoroughly investigated by a variety of techniques – static, dynamic, and electrophoretic light scattering, transmission electron microscopy, and atomic force microscopy. The size and molar masses of the particles as well as other parameters such as aggregation number and number of oligonucleotide strands per particle are significantly larger than those, reported for metal-free spherical nucleic acids. Formation of superaggregates of smaller individual micelles or nonmicellar assemblies by hydrophobic interactions between the synthetic polymer chains and "sticky" interactions between oligonucleotides such as base pairing, π-stacking, hydrogen bonding is anticipated. The "sticky" interactions are counterbalanced by repulsion between the negatively charged oligonucleotide strands thus providing colloidal stability of the structures. The surface density of the oligonucleotide strands in the shell implies that the latter are in a random coil (mushroom) conformation rather than in a fully extended, brush regime. The hallmark properties of the prototypical spherical nucleic acids – non-toxicity and biocompatibility, increased cellular uptake without the need of transfection agents, enhanced nuclease stability – are also exhibited by the novel constructs despite of the differences in size, morphology, and structure.

15. Pavel Videv, Nikola Mladenov, Tonya Andreeva, **Kirilka Mladenova**, Veselina Moskova-Doumanova, Georgi Nikolaev, Svetla D. Petrova, Jordan A. Doumanov, **Condensing Effect of Cholesterol on hBest1 / POPC and hBest1 / SM Langmuir Monolayers** 2021 Condensing Effect of Cholesterol on hBest1/POPC and hBest1/SM Langmuir Monolayers; Membranes, Volume 11, Issue 1, 52

Summary

Human bestrophin-1 protein (hBest1) is a transmembrane channel associated with the calcium-dependent transport of chloride ions in the retinal pigment epithelium as well as with the transport of glutamate and GABA in nerve cells. Interactions between hBest1, sphingomyelins, phosphatidylcholines and cholesterol are crucial for hBest1 association with cell membrane domains and its biological functions. As cholesterol plays a key role in the formation of lipid rafts, motional ordering of lipids and modeling/remodeling of the lateral membrane structure, we examined the effect of different cholesterol concentrations on the surface tension of hBest1/POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) and hBest1/SM Langmuir monolayers in the presence/absence of Ca2+ ions using surface pressure measurements and Brewster angle microscopy studies. Here, we report that cholesterol: (1) has negligible condensing effect on pure hBest1 monolayers detected mainly in the presence of Ca2+ ions, and; (2) induces a condensing effect on composite hBest1/POPC and hBest1/SM monolayers. These results offer evidence for the significance of intermolecular protein–lipid interactions for the conformational dynamics of hBest1 and its biological functions as multimeric ion channel

16. Pavel Videv, **Kirilka Mladenova**, Svetla Petrova, Jordan Doumanov, **STRUCTURE AND FUNCTION OF hBEST1, EXPRESSED IN MDCK II CELLS**, глава от книга, ISSN 1314-3425, p. 387-395, PKP-Print, Глава от Книга,регистрирана в НАЦИД

Summary

Bestrophinopathies are ocular diseases caused by mutations in the human bestrophin-1 (hBest1) - a transmembrane protein and Ca2+-activated chloride channel, mainly expressed in retinal pigment epithelium cells. Its important biological functions range from chloride ions transport to transport of neurotransmitters such as Glu and GABA in the nervous system, involvement in the pathology of brain (Alzheimer's, Parkinson's diseases), regulation of cell volume, regulation of calcium levels and kinetics of volt-dependent channels. Our recent investigations on hBest1 structure-function relationship are concentrated on its interactions with phospholipid monolayers and model membrane systems in order to study Best disease pathogenesis and improve the quality of life in affected individuals.

17. **Mladenova K**., Doumanov J., Petrova S., Videv P., Moskova-Doumanova V., Topouzova-Hristova T., Bakardzhiev P., Toncheva-Moncheva N., Rangelov S., **Biological evaluation of novel amphiphilic nucleic acid - polymer nanoparticles in eukaryotic cells**, FEBS OPEN BIO, vol:11, issue:Suppl. 1, 2021, pages:261-261, Ref, Web of Science

Summary

Polymer-based gene delivery systems are globally studied, as safer and less immunogenic substitutes to viral systems. However, as nucleic acids (NAs) are stable and hydrophilic molecules, and their intracellular delivery and targeting rely on secondary carriers. In this work we are presenting the biological effects of novel nucleic acid-polymer conjugates (NAPCs) on two cell lines – A549 and HepG2. NAPCs are composed by oligonucleotides functionalized with different polyethers such as poly(ethoxyethyl glycidyl ether) and polyesters such as poly(e-caprolactone). The NAPC assemblies were found to carry thousands of oligonucleotide strands per particle. Using MTT and crystal violet assays we investigate cytotoxicity of NAPCs applied on cells at different concentrations. The effect on cell viability was determined by incubating the cells for 48 h with nanoparticles in a concentration-dependent manner. NAPCs behavior into cells was investigated by microscopy observations after Neutral red staining and SYBR Green I staining (Previously published in: Bakardzhiev P et. al. (2020) European Polymer Journal, 131). Our results suggest that the biological properties of the NAPCs include low toxicity and rapid cellular uptake without the need of other transfection agents. We conclude that these novel NAPCs are promising carriers for the delivery of DNA with potential for biomedical applications. Acknowledgments: This work was supported by grant DN 19/8-2017 from Bulgarian National Science Fund.