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Development of methods for suppression of non-wished viral genes and for design of new anti-viral vaccines

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Design of recombinant gene/DNA constructs, containing one or more of the respective tested gene(s) of interest and/or siRNAs, directed to specific target gene(s) in the viral particle, including against SARS-CoV-2/COVID-19, is necessary. Taking in consideration the proved activated formation of thrombs on the influence of Spike (S) protein of virus strain SARS-CoV-2, but also recently proved activated formation of characteristic for dementias amyloid-like plaques in the brain by the nucleocapsid (N) protein of the same virus, it is necessary to be designed molecular vaccines (which could be with DNA-, RNA- or protein nature) against other virus proteins, as for instance, against viral envelope (E) protein and/or against virus membrane (M) protein or against virus, different of the virus S protein, as well as boosting with previously designed specific siRNAs against virus gene, coding virus proteins S and N. Also, adequate cell and/or humoral immune response against side effects, both in vitro and in vivo, should be provided. Sub-populations of laboratory-incubated mammalian cells were transfected with previously designed recombinant gene constructs, based on the DNA-genome of Adeno-Associated Virus (AAV – Parvoviridae family). Other in vitro-cultures of mammalian cells was inoculated with low initial infectious titers (high initial dilutions of viral suspensions) of vaccine avipoxviral strains (Poxviridae family). The monolayers of the inoculated cells were then freezed at −800C in the presence of cryo-protector Dimethylsulfoxide (DMSO), subsequently thawed and re-incubated. As a source of the extra-cellular virus forms served the centrifuged and filtrated cultural fluids, and of their intra-cellular forms - scraped-off cellular monolayers. Then, de novo-seeded cultures of mammalian cells were inoculated with the so prepared intra- and extra-cellular forms of the vaccine viral strains. Presence and expression of the inserted copy of the respective gene of interest was observed in separate sub-populations of mammalian cells, transfected by based on AAV DNA-genome recombinant gene constructs, containing it. The titers of the intra-cellular forms of both vaccine avipoxviral strains were significantly higher compared with these of their extra-cellular forms. After the 24-th hour p. i., increase in the titers of both forms of the viral strains was established. The probable reason was the proved in the scientific literature changed properties of many membrane molecules in these conditions, but also with the influence of many inter-molecular interactions. These features could be explained with the transition of the extra-cellular forms of both strains to their in intra-cellular forms. Furthermore, a possibility for transfer of nucleotide (DNA- and/or RNA-) fragments from virus to cellular genome, as well as in the opposite direction (from cellular to viral genome), influence of organic detergents as DMSO plus availability of drastic temperature changes, was suggested, which was in confirmation with the literature findings. Additionally, a possibility for production of membrane receptor glycoproteins by non-myeloid and non-lymphoid cellular types on the influence of viruses or viral antigens, of malignant cells/antigens, etc., was proposed.

Biography

Iskra Sainova, Research assistant- PhD at the Institute of Experimental Morphology and Anthropology with Museum to Bulgarian Academy of Sciences (IEMAM-BAS) in Sofia, Bulgaria. And her Research Staff Position in: Laboratory cultivation of embryo and adult stem/progenitor cells; Cell differentiation of embryo and of adult stem/progenitor cells; Influence of growth factors, cytokines and other molecules, on the morphology, growth, proliferation and differentiation of normal and malignant cells in vitro and in vivo; Education: Assistant professor-PhD at the Institute of Experimental Morphology, Pathology and Anthropology with Museum (IEMPAM) to Bulgarian Academy of Sciences (BAS) in Sofia, Bulgaria, Project title: “Development of methods for derivation of different normal mature cell lineages, by appropriate laboratory incubation of stem/progenitor cells from different sources”.