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Animal Posters

A-2000

Signaling Pathways of Modulated Electro-hyperthermia Induced Tumor Cell Killing. ENIKŐ MAJOR, Andrea Balogh, Balázs Besztercei, Anett Benedek, and Zoltán Benyó. Institute of Translational Medicine, Semmelweis University, Budapest, HUNGARY. Email: major.eniko@med.semmelweis-univ.hu

Modulated electro-hyperthermia (mEHT) is a novel, non-invasive, tumor-selective modality in cancer treatment. In clinical practice, mEHT is used complementary to chemo- and radiotherapy. The objective of the present study was to examine the effect of mEHT on B16F10 mouse melanoma cell line in vitro and in vivo. For in vitro studies, B16F10 cells were grown on coverslips, treated with mEHT using LabEHY 100 (Oncotherm Ltd.) at 42 °C. In combined protocols, after 60 minutes mEHT, cells were treated with dacarbazine (40 μM), paclitaxel (40 nM), or nutlin-3a (10 μM). Changes in gene expression, viability, and invasiveness were measured. For in vivo studies, B16F10 cells were injected subcutaneously into C57Bl/6 mice and the tumors were exposed to 42 °C for 30 minutes with mEHT every second day for three times. Tumors were removed 48 hours post-treatment for further analysis. In vitro, the upregulation of pro-apoptotic genes Puma, Bak-1, Bax, and downregulation of pro-survival genes XIAP, Bcl-2, Bcl-XL were induced by mEHT. In combination with chemotherapy, mEHT augmented the cell-killing effect of dacarbazine or nutlin-3a whereas failed to affect paclitaxel-induced cell death 48 hours post-treatment. In the tumors treated in vivo, mEHT induced a massive DAMP release, the upregulation of p21 and p27 as well as increased macrophages infiltration. Taken together, our results demonstrate that hyperthermia-induced nuclear translocation of p53 leads to cell cycle arrest and cell death reflected in decreased cell viability. Hyperthermia may disrupt tumor membranes allowing greater permeability of chemotherapeutics to access the cell resulting in a greater therapeutic effect. This study was supported by NVKP 16–1-2016–0042 grant and by the Richter Gedeon Talentum Foundation.

A-2001

Toxic Assessment of Environmental Contaminants Using a Novel *In Vitro* High Throughput Bioassay Screening System of Gap Junctional Intercellular Communication. BRAD L. UPHAM[1], Lizbeth Lockwood[1], Jamie Liebold[1], Joo Hye Yeo[2], Jinu Lee[2], Alison K. Bauer[3], Erika Lisabeth[4], and Richard Neubig[4]. [1]Department of Pediatrics and Human Development, Michigan State University, East Lansing, MI 48824; [2]College of Pharmacy, Yonsei Institute of Pharmaceutical Sciences, Yonsei University, Incheon 21983, SOUTH KOREA; [3]Department of Environmental and Occupational Health, University of Colorado-Denver, Aurora, CO 80045; and [4]Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824. Email: upham@msu.edu

The selection of gap junctional intercellular communication (GJIC) as a toxicological endpoint is a significant step in developing a systems-based *in vitro* model, as this biological phenomenon is crucial for integrating signaling mechanisms within cells with that of neighboring cells in a tissue and is an important early-stage event in abnormal cell proliferation within tissues. Most *in vitro* assessments of GJIC tend to be problematic in developing high throughput screening (HTS) assays. We present our latest results using a novel HTS system developed with the F344 WB cell line, which is an excellent *in vitro* cell model of liver oval cells, a bipotent stem/progenitor cell that give rise to hepatocytes and hepatic biliary duct cells and self-renew. This assay depends on a subset of donor are stably transfected with the iodide transporter gene and a subset of receptor cells stably transfected with the yellow fluorescent protein (YFP) gene. The addition of iodide initiates the bioassay by entering the donor cells via the iodide transporter, and then transfers through gap junctions to the receptor cells, in which iodide quenches the YFP-fluorescence. Closed or partially closed gap junction channels prevents or partially prevents quenching in the receptor cells from iodide. Multiple well plates are used, and fluorescent plate readers measure the fluorescence,

which makes this bioassay quite amendable to HTS. We have results on the effects of a select set of polycyclic aromatic hydrocarbons PAHs and select set of perfluoroalkyl and polyfluoroalkyl substances (PFAS) using this HTS assay and compared the results to our standard scalpel load dye transfer assay. *Research supported by the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health (NIH) under Award Number R21ES031345. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH-NIEHS.*

A-2002

Effects of Sodium Butyrate and D- and L-lactate on Rainbow Trout Intestinal Epithelial Cells. DAYLAN PRITCHARD[1], Eryn Braley[1], Patrick G. Pumputis[2], Vivian R. Dayeh[2], Niels C. Bols[2], and Lucy E. J. Lee[1]. [1]Department of Biology, University of the Fraser Valley, Abbotsford, BC, CANADA and [2]University of Waterloo, Department of Biology, Waterloo, ON, CANADA. Email: daylan.pritchard@student.ufv.ca

Products of microbial fermentation in the vertebrate gastrointestinal (GI) tract act on GI epithelial cells to promote health in humans and in farm animals. Among the products, butyrate is the most commonly examined short chain fatty acid (SCFA), but microbial fermentation also produces the closely related D- and L-lactate. How these compounds act in fish is of interest in evolutionary physiology and in aquaculture, where they are being considered as dietary supplements, but little is known of their actions on teleost cells. The rainbow trout intestinal epithelial cell line, RTgutGC, was used to study the effect of butyrate and lactate on epithelial integrity and wound repair. This was done in complete growth medium, L15 with 10% fetal bovine serum (L15/FBS), and in different kinds of nutritional deprivation: no serum (L15), no amino acids and vitamins (L15/ex) and no nutrients (L15/salts). Over 7 days, RTgutGC monolayers remained intact in all four media, but cell migration only occurred in L15 and L15/FBS and wound healing was completed only in L15/FBS. In all 4 media, RTgutGC monolayers remained intact with 0.1 to 2.0 mM butyrate. Butyrate at 0.1 to 2 mM stimulated cell migration in L15 but neither modulated the migration that occurred in L15/FBS nor made migration possible in L15/ex and L15/salts. At higher concentrations, butyrate caused morphological changes, F-actin disruption, and monolayer deterioration. This cytotoxic response was most pronounced in the presence of FBS. Similar results were achieved with lactate. Overall the results suggest that there is a narrow concentration range where the fermentation products might be beneficial to rainbow trout intestinal health.

A-2003

Explant Cultures of Rainbow Trout Olfactory Rosettes (ORs): Cell Outgrowth, Degeneration, Propagation, and Characterization. R. GOLDBACH[1], J. B. Lee[1], N. C. Bols[2], and L. E. J. Lee[1]. [1]University of the Fraser Valley, Department of Biology, 33844 King Rd, Abbotsford, BC V2S 7M8, CANADA and [2]University of Waterloo, Department of Biology, 200 University Ave W, Waterloo, ON N2L 3G1, CANADA. Email: ryan.goldbach@student.ufv.ca

Cell cultures were initiated from rainbow trout olfactory rosettes (ORs). Fish ORs consist of folded sheets of olfactory mucosa (OM) attached to a central support (raphe). The OM is a pseudostratified epithelium, in which leucocytes are interspersed, with an underlying lamina propria (LP), in which leucocytes and melanophores are found. Most but not all of the epithelium has olfactory sensory neurons (OSNs) and is termed the olfactory epithelium (OE). After being cut into pieces, ORs were incubated in L15 with fetal bovine serum in plastic culture vessels to which they adhered. Over several days epithelial cell sheets migrated out from OR fragments, while melanophores largely remained within them. Time lapse microscopy revealed the steady expansion of epithelial sheets until spreading abruptly halted and even retreated. This was due to the cells at the outer edges synchronously taking on the morphological appearance of apoptotic cells and degenerating. Possibly they were OSNs, responding to axotomy. Yet, epithelial sheet migration from OR fragments continued, pushing out fronts of apoptotic bodies. With longer culturing, episodic epithelial degeneration stopped but migration from ORs continued, leading after several months to cultures that could be sub-cultivated and developed into epithelial cell lines (RTolfs). The cell lines possibly arose from OE stem cells, globose basal cells (GBC) and horizontal basal cells (HBC), or LP mesenchymal stem cells (LP-MSCs). Although predominantly epithelial-like, RTolf cultures through early passages consistently contained some dendritic cells, based on their morphology, movement and phagocytic activity. Further understanding of these cultures should make them useful for studying ORs in fish health and disease.

A-2004

The Investigation of SNP in SOCS2 Gene Associated with Mastitis Resistance, Milk Composition, and Quality in Awassi Sheep. M. UL HASAN and A. Ceyhan. Nigde Omer Halisdemir University, TURKEY. Email: mrmubeen.hassan@gmail.com

Milk production is a very important trait in the livestock industry. Milk quality and milk fat content, protein

composition, and vitamin contents are important for milk consumption all over the world. Mastitis disease of the udder in livestock has caused a considerable loss to the milk production industry worldwide. The present study is focused on milk quality, milk composition, and mastitis disease in the Turkish Awassi sheep breed. In comparison to cattle and goat, sheep milk has unique nutrient content. Additionally, the fat content, mineral and vitamin content, and total solid are more in sheep milk as compared to goat and cattle. The presence of magnesium, phosphorus, calcium and phosphorus vitamins A, B, and E make sheep milk and its products more beneficial for health. The suppressor of the Cytokine signaling 2 (SOCS2) gene has a role in milk production and milk quality in cattle, sheep, and goats and in controlling mastitis disease by activating the immune system against pathogens (Oget et al., 2019). In this study, we will check the polymorphism in the SNP present in the SOCS2 gene by using Taq-Man Real-time PCR Assay and study their relationship with milk quality, milk composition, and providing resistance against mastitis. Moreover, we didn't find any investigation from Turkey relating to the effect of the suppressor of cytokine signaling 2 gene against mastitis and milk composition. The objective of the study is to find the polymorphism of the SNP (rs868996547) in the cytokine signaling 2 gene associated with resistance against mastitis disease. The resistance against mastitis provided by the polymorphism in the SOCS2 gene has been reported previously. Moreover, the present study will also record milk fat content, protein composition in milk to check the effect of the polymorphism on the important milk traits.

A-2005

Antioxidant, Proliferative and Migrative Potential of the *Maclura tinctoria* on *In Vitro* Analyses. REGGIANI V. GONCALVES[1,2], Eduarda P. Costa[2], Mariaurea M. Sarandy[2], Rômulo D. Novaes[3], Jade Schlam[1], and Debora Esposito[1]. [1]Plants for Human Health Institute, North Carolina State University, NCRC, Kannapolis, NC and [2]Department of Animal Biology, UFV, Viçosa, BRAZIL; Federal University of Alfenas, Alfenas, BRAZIL. Email: rvilela@ncsu.edu, daesposi@ncsu.edu

The oxidative damage produced by free radicals has been studied and confirmed in many chronic diseases. During the skin wound healing process, the trauma in the dermis promotes a release of free radicals, and Reactive Oxidative Species (ROS) that are responsible by to keep the inflammatory process delaying the wound healing. Currently, some natural products have antioxidant activity and protect the human body from free radicals action, preventing oxidative stress in tissue. In this context is *Maclura tinctoria* (M. tinctoria), knowledge like "wild mulberry", which is the Moraceae family, used in Brazilian cuisine. It presents medical propriety like healing, anti-rheumatic, anti-inflammatory, antimalarial, antifungal, and anti-HIV. These curative proprieties of *M. tinctoria* should be associated with phenolic compounds present in this extract (isoflavones and prenylated flavones). Therefore, this study aims to investigate the antioxidant, proliferative and migratory potential of the *M. tinctoria* leaves extract on RAW264.7 macrophages. We were testing antioxidant dichloromethane extracts on the 25 and 50 µg/mL concentrations using DPPH, and cell viability after H2O2 exposition and Catalase enzymes quantification. The scratch assay was done to analyze the cell migration. The *M. tinctoria* extracts showed high antioxidant activity, cytoprotective, proliferative, and migrative capacity in the 25 µg/ml concentration. This extract promotes benefits to the oxidative balance and healing process on *in vitro* models by the modulation of the antioxidant enzymes and cryoprotection after H2O2 exposition, besides stimulating the cell migration during the inflammatory phase on the wound healing process. These effects can be attributed to the high content of phenolic compounds in the extract.

A-2006

Effect of Phytocannabinoids and Anti-aging Drugs on Skin Rejuvenation During Aging and Oxidative Stress. MARTA GERASYMCHUK, Gregory Robinson, Olga Kovalchuk, and Igor Kovalchuk. Department of Biological Sciences, University of Lethbridge, Lethbridge, AB, CANADA. Email: Marta.Gerasymchuk@uleth.ca

The therapeutic use of *Cannabis* plant has been known over millennia. *Cannabis Sativa* L. produces over 100 phytocannabinoids, including delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). Cannabinoids is effective in numerous pathological conditions such as fibrosis and skin disorders. However, there is a lack of scientific knowledge about the anti-aging and rejuvenation properties of cannabinoids. The objective is to analyze the overarching patterns to determine whether phytocannabinoids, anti-aging drugs, and their combinations have anti-aging effects through inhibiting inflammatory processes, preventing aging-related nuclear structure changes, and/or delaying the senescence of skin cells. Human skin fibroblasts (CCD-1064Sk, ATCC) and foreskin fibroblasts (BJ-5ta, hTERT-immortalized cell line) were treated with hydrogen peroxide (H2O2) to reproduce cellular senescence, and subsequently treated with THC, CBD, anti-aging drugs, and their combinations. Cellular and molecular mechanisms of aging were determined by β-galactosidase senescence assay, MTT colorimetric cell viability assay, western immunoblotting, reverse transcription-polymerase chain reaction, and nuclear DAPI staining. Fibroblasts exposed to H2O2 and treated with THC,

CBD, anti-aging drugs demonstrated significantly decreased molecular and cellular senescence markers than untreated cells. Collagen genes associated with senescence, were downregulated. Nuclear staining showed significant structural changes of nuclei in aged fibroblasts compared to those treated with THC/CBD. Phytocannabinoids exert an anti-aging effect on human skin fibroblasts and stimulate rejuvenation effects by increasing cell longevity, stimulating collagen production, and preserving nuclear structural integrity.

A-2007

Novel Mammalian Fibroblast Cell Culture Media Technique for Ultraviolet Cell Reduction. J. HOEGER. University of Dubuque, Applied and Natural Sciences, 2000 University Ave, Dubuque, IA 52001 and Tri-Vet Associates, 210 Beltline Rd, Dyersville, IA 52040. Email:jhoeger04@gmail.com

Carcass bruising from horns costs the Canadian cattle industry $10 million a year (Anderson, 2012). It is vital to develop safe and cost-effective advancements in dehorning procedures. Removal of horns raises risk of discomfort and pain to the animal; utilizing ultraviolet (UV) light as treatment can be effective when compared to current methods. The objective was to develop a novel media and technique to effectively culture horn producing cells for in vitro UV experimentation. Three media variations failed due to lack of cell reproduction and presence of fungal or bacterial colonies. Horn producing cells demonstrated an average increase of 31.39% in a novel media mixture of 20 ml Ham's F12, 2% Amphotericin B from Streptomyces sp., and 1% penicillin. A UV prototype, developed in previous research was improved to guarantee isolated UV treatment of horn producing cells. Varying times were adapted for ultraviolet treatment. Horn producing cells were cultured in this media and evaluated by cell count using ImageJ analysis of digital images. Statistical analysis found significant relationships between cell death or reduction and run time of the UV lamp. As light run time increased, mitosis decreased. Ultraviolet light substantially reduced mitosis as compared to controls without UV exposure. Prototype design and implementation were effective for isolated treatment of cells in novel media.

A-2008

The Investigation of LNP-siRNA Formulations for Rainbow Trout Fish Cell Lines Through the Examination of Cellular Uptake and Reporter Gene Knockdown *In Vitro*. HEATHER M. KELLY[1], Sam Chen[2], Yuen Yi C. Tam[2], Natallia Varankovich[3], Sikander Gill[3], Lucy E. J. Lee[1], and Justin B. Lee[1]. [1]Department of Biology, University of the Fraser Valley, Abbotsford BC, CANADA; [2]Integrated Nanotherapeutics, Burnaby BC, CANADA; and [3]Lumex Instruments, Mission BC, CANADA. Email: Heather.Kelly1@student.ufv.ca

Lipid nanoparticles (LNPs) encapsulating nucleic acids such as mRNA and siRNA have been used in many types of research and clinical settings as they allow for non-viral gene delivery. For example, siRNA has been used in eukaryotic cells to induce RNAi and cause subsequent degradation of target mRNA. Although there has been plenty of research done on the therapeutic use of LNP systems in humans, there has been little use of LNPs or other nanoparticles to induce gene expression or gene silencing in aquaculture. Fish mortality due to infectious diseases is a major concern in the aquaculture industry, but the reliance on antibiotics can lead to complications such as antibiotic resistance in both fish and humans. LNPs encapsulating nucleic acids such as siRNA (LNP-siRNAs) can potentially be utilized as replacements for these antibiotics. The cell lines used in this research include three established rainbow trout cell lines: a gill epithelial cell line (RTgill-W1), a gut epithelial cell line (RTgutGC), and a macrophage cell line that stably expresses Turbo green fluorescent protein (RTS11-GFP). The present research aims to examine cellular uptake, gene expression, gene silencing, and cytotoxicity through the use of fluorescently Dil-labeled LNP-siRNA formulations. Preliminary results indicate that Dil-LNP-siRNAs are successfully taken up by the tested rainbow trout cell lines and that there are no significant cytotoxic effects. GFP-DsiRNA was also transfected into the RTS11-GFP cell line and RT-PCR was used to verify gene expression and gene silencing prior to LNP encapsulation.

A-2009

Investigation of Endocytic Mechanisms of Lipid Nanoparticles (LNP-siRNA Systems) into Rainbow Trout Fish Cells *In Vitro*. K. N. VAN WOUDENBERG[1], S. Chen[2], Y. Y. C. Tam[2], L. E. J. Lee[1], and J. B. Lee[1]. [1]Department of Biology, University of the Fraser Valley, Abbotsford, BC, CANADA and [2]Integrated Nanotherapeutics, Burnaby, BC, CANADA. Email: katriana.vanwoudenberg@student.ufv.ca

Lipid nanoparticles (LNPs) are small particles (~ 50–60 nm in diameter) used to encapsulate drugs or nucleic acids (such as siRNA) for delivery to cells or tissues. Endocytosis is a regulatory process through which cells internalize particles in membrane vesicles. RTgill-W1 and RTgutGC are well-characterized, metabolically active rainbow trout (*Oncorhynchus mykiss*) epithelial cell lines used for *in vitro* studies. The aim of this research was to establish the minimum time required for endocytosis of LNP-siRNAs occur in RTgill-W1 and RTgutGC cells, and to determine which endocytic mechanism fish cells use to take-up LNPs. DiI-fluorescent labeled LNP-siRNA particles were used to assess cellular uptake in RT cells using 2, 4, 6, 8, 12, 16, and 24 hour time points. RTgutGC began to show uptake

by 2 hours RTgill-W1 began to show uptake by 4 hours and both cell lines had significant uptake by 8 hours. A variety of inhibitors were used to study the mechanism of endocytosis including chlorpromazine (inhibits clathrin-mediated endocytosis), nystatin (inhibits caveolae-dependent endocytosis), genistein (inhibits caveolae-mediated endocytosis) and amiloride (inhibits micropinocytosis). To determine the mechanism(s) of endocytosis, cells were pre-treated with inhibitors and DiI-fluorescent LNP-siRNAs. Results forthcoming on LNP-siRNA cellular uptake following endocytic inhibition.

A-2010

Mapping Interneuron Migration During Late Neurodevelopment in the Piglet Brain. K. NAKAMURA and M. Paredes. Department of Neurology, University of California, San Francisco, CA. Email: keira.nakamura@gmail.com

Inhibitory neurons (interneurons) are a neuronal subpopulation that has been strongly implicated as causal to neurodevelopmental disorders, conditions such as autism spectrum disorder and epilepsy. Recent evidence has shown robust interneuron migration into the frontal cortex of the human brain during the first months of life from a region known as the ganglionic eminence. This suggests that the human cortex, the brain region associated with higher cognitive functions, remains dynamic even after birth. The migration patterns of these late-migrating interneurons are not well understood or how they might contribute to the final stages of cortical development. Our gaps in knowledge arise from the rarity of samples from this late neurodevelopmental stage and limitations in current animal models, such as the rodent brain. My study will implement a novel animal model, the domestic pig brain, to study the molecular diversity and organization of these cells and generate a map of interneuron migration in the early piglet cortex. Understanding this normal late cortical development is critical to identifying disruptive processes that could lead to neurodevelopmental diseases.

A-2011

Exosomes Enriched in Angiogenic/Growth Factors Are Released from Human Coronary Smooth Muscle Cells Grown on 3D Silk Fibroin Nonwovens. UBALDO ARMATO[1,2], Peng Hu[1,3], Anna Chiarini[1], Jun Wu[2], Zairong Wei[3], and Ilaria Dal Prà[1,2]. [1]Department of Surgery, Dentistry, Paediatrics & Gynecology, University of Verona, ITALY; [2]Department of Burns & Plastic Surgery, University of Shenzhen, CHINA; and [3]Department of Burns & Plastic Surgery, ZunYi Medical University, CHINA. Email: uarmato@gmail.com

Our earlier works showed the quick vascularization of *Bombyx mori* 3D silk fibroin nonwoven scaffolds (3D-SFnws) grafted into mouse skin [Chiarini et al. Tissue Eng Part A. 2016, 22:1047–60] and the release of exosomes enriched in angiogenic/growth factors (AGFs) from *in vitro* 3D-SFnws-stuck human dermal fibroblasts (HDFs) [Hu et al. Burns Trauma. 2021, 9:tkab003]. In this work we investigated whether coronary artery adult human smooth muscle cells (AHSMCs) might also release AGFs-enriched exosomes when cultured on 3D-SFnws *in vitro*. Media with exosome-depleted FBS served for AHSMCs and human endothelial cells (HECs) cultures on 3D-SFnws or polystyrene plates. *D*-glucose and dsDNA assays and double-antibody arrays were used to assess growth, metabolism, and activation of intracellular TGF-b and NF-kB signalling pathways. AGFs conveyed by $CD9^+/CD81^+$ exosomes released from AHSMCs were analysed via double-antibody arrays and their angiogenic power evaluated on HECs *in vitro*. Our results show that AHSMCs on SFnws *vs*. polystyrene proliferated and consumed *D*-glucose more intensely and showed a stronger phosphorylation, i.e. activation of TAK-1, SMAD-1/-2/-4/-5, ATF-2, c-JUN, ATM, CREB; and an IkBaphosphorylation/inactivation¾overall consistent with a proliferative/secretory rather than contractile phenotype. SFnws-stuck AHSMCs also released exosomes richer in IL-1a/-2/-4/-6/-8; bFGF; GM-CSF; and GRO-a/-b/-g, which strongly stimulated HECs' growth, migration, and the assembly of endothelial tubes/nodes *in vitro*. Altogether, the intensified AGFs exosomal release from 3D-SFnws-attached AHSMCs and HDFs could advance the cellular colonization, vascularization, and take *in vivo* of silk fibroin scaffold supported grafts—all noteworthy assets for prospective clinical applications.

A-2013

Oligonucleotide Vaccines: The Joker in the Vaccine Deck. VOLODYMYR V. OBEREMOK, Oksana A. Andreeva, Ilya A. Novikov, and Kateryna V. Laikova. Laboratory of Molecular Genetics and Biotechnology, V. I. Vernadsky Crimean Federal University, Simferopol, Crimea, RUSSIA. Email: genepcr@mail.ru

In March 2020, our research group, which has been working with oligonucleotide constructs for about 15 years, thought about the possibility of using phosphorothioate oligonucleotides to create vaccines against the coronavirus. We did a preliminary review of the concept of oligonucleotide vaccines in the journal of Inflammation Research (https://doi.org/10.1007/s00011-020-01,352-y). We continue to research and test two main types of oligonucleotide constructs, semi-natural and natural. Studies

carried out over the past few decades have accumulated sporadic but convincing data on the possibility of using nucleic acids as an active immunogen, but for this to succeed, it is necessary to set certain parameters: the nucleic acids must be single-stranded rather than double-stranded;-the nucleic acids must have a permanent multi-dimensional nanostructure; the nucleic acids must have a unique sequence that is not present in the host genome. In our experiments with SARS-CoV-2, a semi-natural vaccine of the La-S-so (lamellar anti-SARS-CoV-2 sulfur-containing oligonucleotide) type was found to have a moderate immune response, expressed by formation of specific antibodies. In addition, studies of the La-S-so vaccine have been conducted in humanized mice with the human ACE2 receptor. By day 30 of the experiment, it was shown that, while all the vaccinated animals were alive and beginning to recover, all the control mice had died. The morphological parameters of the lung parenchyma were comparable to those of intact animals. Two obstacles stand in the way of the development of oligonucleotide vaccines: the absence of knowledge about a) antibodies capable of penetrating cells and attacking the unique nucleic acid sequences of RNA viruses, and b) antigen presentation ability of dendritic cells with respect to nucleic acids. At the same time, there is no doubt that oligonucleotide vaccines based on highly conserved regions of the RNA virus genomes have great potential in the prevention of viral diseases, despite the fact that they do not yet fit within the framework of a modern textbook on immunology.

A-2014

The Designing of *Bacillus Anthracis* Full-size Recombinant Protective Antigen with Modifications Improving Its Stability. EKATERINA M. RYABCHEVSKAYA, Dmitriy L. Granovskiy, Ekaterina A. Evtushenko, Peter A. Ivanov, Olga A. Kondakova, Nikolai A. Nikitin, and Olga V. Karpova. Lomonosov Moscow State University, Department of Virology, Leninskie Gory 1–12, 119234, Moscow, RUSSIA. E-mail: eryabchevskaya@gmail.com

Anthrax is a serious infectious disease which is caused by a bacterium *Bacillus anthracis*. Its spores are highly resilient and are one of the most likely bioterrorist agents. Therefore, the development of an effective anthrax vaccine is an urgent task given shortcomings of licensed vaccines. Recombinant *B. anthracis* protective antigen (rPA) is the most common basis for modern anthrax vaccine candidates. However, there is a serious problem with its low stability. Moreover, having being adsorbed on aluminum hydroxide, an adjuvant used in almost all currently developing anthrax vaccines, rPA rapidly loses its ability to induce neutralizing antibodies. One of the ways known to increase rPA stability is cleavage sites inactivation; the other one is the replacement of deamidation-prone asparagines by glutamines. Here we for the first time combined both variants of these modifications and generated modified full-size rPA (rPA83m) with inactivated proteolysis sites (^{162}NSRKKR^{167} was replaced by ^{162}QSSNKE^{167} in furin cleavage site and ^{313}FF^{314} was deleted in chymotrypsin cleavage site) and the most deamidation-prone Asn713 and Asn719 substituted by glutamines. The stability of rPA83m was considerably higher comparing to non-modified rPA. Furthermore, the compositions of rPA83m with spherical particles (SPs), which are tobacco mosaic virus-based platform-adjuvant, were obtained. SPs-rPA83m compositions were shown to interact with PA-specific antibodies. Considering previously demonstrated SPs ability to increase non-modified rPA stability, we suggest that SPs will also be able to additionally stabilize rPA83m within vaccine formulation. This work was supported by the Russian Science Foundation (Grant number 18–14-00,044).

A-2015

The Phosphorothioate Antisense Oligonucleotide Hush-11 Shows Substantial Antimelanoma Effect in Mice. KSENIYA A. YURCHENKO, Kateryna V. Laikova, Volodymyr V. Oberemok, Ilya A. Novikov, Alyona A. Yurchenko, Tatyana P. Makalish, Irina I. Fomochkina, and Anatoly V. Kubyshkin. V. I. Vernadsky Crimean Federal University, Simferopol, RUSSIA. Email: yurchenkokseniya28@gmail.com

Skin cancer remains the leader among all tumors in terms of incidence; among these, the deadliest is melanoma. The need to find new therapeutic agents to combat skin cancer is urgent. In our opinion, antisense oligonucleotides aimed at targeted suppression of the genes in cancer cells responsible for survival offer real hope for a treatment that will eliminate cancer cells near the tumor focus both before and after surgery. Hush-11 is antisense oligonucleotide (5'-AGC-TAT-CTC-CG-3') for glucose-6-phosphate dehydrogenase gene (G6PD). A decrease in tumor size in mice was found in response to the use of Hush-11. On average, tumor size decreased 15% per day in the group treated with Hush-11 for 7 days, while in the control group the size of the tumor increased by 6% per day. The average tumor size in the groups at the beginning of the experiment was 0.29 ± 0.04 cm^2; on the 7th day of the experiment, significant differences were observed between the average tumor area of the control group (0.35 ± 0.06 cm^2) and the group treated with Hush-11 (0.15 ± 0.05 cm2) ($p < 0.05$). Random oligonucleotide OligoA11 (5'-AAA-AAA-AAA-AA-3') did not show any significant effect on melanoma growth compared with the control (0.4 ± 0.11 cm^2). The results of an immunohistological

examination for the Bcl-2 marker showed that 11.8 ± 0.84 cells were detected in the field of view for the Hush-11, which was 40.67% more than for the control group, where 7 ± 0.47 cells were detected in the field of view ($p < 0.01$). For the FAS marker, 10.2 ± 1.06 cells were detected in the field of view for the Hush-11, which was 64.7% more than for the control group, where 3.6 ± 0.45 cells were detected in the field of view ($p < 0.01$). An interesting fact was noted that in the Hush-11 group, 2.5 fewer amitosis were observed compared to the control group ($p < 0.05$). Thus, inhibition of G6PD expression with antisense oligonucleotides is an effective tool for the treatment of melanoma, but it should be noted that this method is most suitable for local treatment, since central administration of phosphorothioate oligonucleotide may lead to a large number of side effects.