Translational Research and Nano – Biotechnology Oral Presentation Senior Scientists' Forum

Quantum dot multiplexing technology for cancer diagnosis and prognosis

[1]Zhau Haiyen E., [1]Hu Peizhen, [2]Zhu Guodong, [1]Wang Ruoxiang, [3]Berel Dror, [3]Rogatko Andre, [4]Luthringer Daniel, [5]Wang Yuzhuo, and [1]Chung Leland W. K.

1Uro-Oncology Research Program,
2Department of Urology, School of Medicine, Xi'an Jiaotong University, Xi'an, China;
3Biostatistics & Bioinformatics, Department of Medicine;
4Department of Pathology, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center; Los Angeles, CA, USA;
5BC Cancer Agency, University of British Columbia, Vancouver, B.C., Canada
Haiyen.Zhau@cshs.org

Background: The standard practice in pathology for cancer diagnosis and detection has been limited to conventional immunohistochemistry (IHC) color imaging coupled with pathological evaluation of tissue or cell specimens. IHC true color imaging cannot detect multiple biomarkers at a time on the same specimen due to the inability to un-mix the signals. A more dynamic detection technology is needed.

Objectives: To develop a multiplexed quantum dot-IHC (mQD-IHC) protocol using QD light-emitting nanoparticles with composition-dependent tunable emission from visible to near infrared to expand the pathology from a primarily diagnostic and prognostic based discipline to the capability of predicting the lethal progression of cancer. Specific aims are: 1) detect the expression/activation of critical cell signaling proteins at the single cell level; 2) image the plasticity of human prostate cancer (PCa), such as the ability to undergo epithelial to mesenchymal transition; and 3) examine the utility of this technology in clinical PCa specimens to determine its ability to invade and to predict the metastatic capability of human PCa.

Results: The co-expression or activation of β2-Microlgobulin (β2-M), phosphorylated cyclic AMP responsive element binding protein (pCREB) and androgen receptor (AR), assigned as a triple-positive, was examined in 10 PCa tissue specimens from patients with known metastatic status. The overall median % triple positive for $\beta2\text{-}M^{+}/pCREB^{+}/AR^{+}$ cells was 51.5%. When stratified by metastasis, there is a significant difference in the % triple positive for the samples with metastatic potential (median = 61%) vs. those without metastatic potential (median = 0%); p=0.01 by a *Wilcoxon rank sum test*. The results were confirmed in 11 PCa bone metastatic specimens.

Conclusion: Additional tissue specimens with confirmed survival data will be analyzed and the cell-signaling-network-based mQD-IHC will be automated by Vectra System in a high throughput manner. By defining the expression/activation of the β2-M/p-CREB/AR and other relevant cancer progression-associated signaling components using the new mQD-IHC detection and image analysis protocol, one could expand the pathology from a primarily diagnostic- to prognostic-based discipline, capable of predicting the lethal progression of PCa *prior to* clinical manifestation of distant metastases.

Persistence of viral RNA in the brain, hearts and pancreas of experimentally infected mice with coxsackieviruses

[1]Bopegamage Shubhada, [1]Motusova Jana, [1]Stipalova Darina, [1]Sojka Martin, [1]Badurova Miriama, [1]Borsanyiova Maria, [1]Marosova Lenka, and [2]Sobotova Zdenka

[1]Department of Virology, Slovak Medical University,
Limbová 12, 83303 Bratislava, Slovak Republic

[2]National Reference Centres, Public Health Office of the Slovak Republic,
Trnavska 52, 82645 Bratislava, Slovak Republic

shubhada.bopegamage@szu.sk

The **aim** of our study was to follow the persistence of viral RNA in selected organs of experimentally infected with coxsackievirus (CV) B strains from different sources such as a patient's sample, an environmental sample and prototype virus strains.
Methods. CD-1 mice were infected with CVB4 diabetogenic strain E2, the prototype strain of CVB5, isolates from treated sewage waste and isolates from patient´s stool sample or CSF identified either as CVB4 or CVB5. The viral RNA was detected by RT-PCR using enterovirus primers specific for the non-coding 5' region.
Results. We observed presence of RNA in the brain and heart of mice infected with isolates from patient´s stool or CSF at day 45 post infection (p.i.).
Conclusion. We conclude that CVB4 and CVB5 persist in the brain and heart after oral infection of CD1 mice. The relevance of viral persistence maybe related viral origin and the genetics.

Acknowledgements: This work was supported by the Norwegian Financial Mechanism, Mechanism EEA and Slovak Government and the State Budget of the Slovak Republic (SK 0082)

Translational Research and Nano – Biotechnology Oral Presentation Young Scientists' Forum

Comparative characteristics action of COX-2 selective inhibition and COX/LOX dual blockage on ulcerogenic processes in gastric mucosa of rats

Fomenko I. S., Sklyarov A. Ya., Bondarchuk T. I., Lesyk R. B.

Danylo Halytskyi National Medical University, Lviv, Ukraine
69, Pekarska St., Lviv, Ukraine, 79010

dr_r_lesyk@org.lviv.net

Compounds that combine COX and LOX inhibition are potential new drugs to treat the inflammation. They act by inhibiting of both PGs and LTs formation, which are involved in the pathogenesis of gastric ulcer disease. Such combined inhibition avoids some of the disadvantages of selective COX-inhibitors. The aim of the research was to compare changes of NO-synthase system and oxidative stress indexes in gastric mucosa of rats under application of COX-2 inhibitor celecoxib and preparations possessing dual COX/LOX inhibition darbufelon and 4-{2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxothiazo-lidin-3-yl]-pyrrolidin-1-yl}-benzene-sulfonamide on the background of gastric ulcer induced by the injection of adrenalin. Ulcerative lesions in gastric mucosa of rats where modeled by intraperitoneal injection of adrenalin (2 mg/kg). Selective COX-2 inhibitor celecoxib, COX-2/5-LOX inhibitor darbufelon and COX/LOX inhibitor 4-{2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxothiazo-lidin-3-yl]-pyrrolidin-1-yl}-benzene-sulfonamide were introduced in a single dose 10 mg/kg (per oz.) on the background of adrenalin injection. Lipoperoxidation processes were evaluated by MDA content, activity of enzymes of the antioxidant protection system was evaluated on basis of determination of SOD and catalase, Griess reagent was used to measure the content of NO. NOS activity was measured by method of Sumbaev.

Under conditions of modeled gastric ulcer, were observed destructive damage of the gastric mucosa, activation of NO-synthases, predominantly iNOS (more than 10 times), accompanied by increase of NO content by 60%, enhancement of lipoperoxidation processes in the gastric mucosa. Celecoxib owing to its anti-inflammatory and antioxidant action reduced changes, caused by injection of adrenalin. It decreased MDA concentration by 78% and activity of iNOS by 57%. Activity of SOD decreased by 34 % as compared with adrenalin action. Dual acting COX-2/5-LOX inhibitor darbufelon intensified ulcerogenic action of adrenalin causing increased area of destructive damage of the gastric mucosa. Concomitantly, indexes of oxidative stress and NO-synthase system under condition of darbufelon action didn't change significantly in comparison with the action of adrenalin alone. Its use as pharmaceuticals needs further investigation and preclinical testing. Dual COX/LOX inhibitor 4-{2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxothiazo-lidin-3-yl]-pyrrolidin-1-yl}-benzene-sulfonamide manifested pronounced cytoprotective effect under conditions of modeled gastric ulcer. It was accompanied by sharp decline of the activity of NO-synthases (iNOS activity decreased more than 2 times) and indexes of oxidative stress.

Anticancer activity of novel drugs delivered by newly synthesized polymeric nanocarriers

[1]Lehka L. V., [2]Panchuk R. R., [3]Mitina N., [3]Skorokhoda T., [3]Moskvin M., [3]Zaichenko O., [4]Havrylyuk D. Ya., [4]Lesyk R. B., [1,2]Stoika R. S.

1 – Ivan Franko Lviv National University, Ukraine
1, Universytetska str., Lviv, Ukraine, 79000
2 – Institute of Cell Biology, NAS of Ukraine, Lviv, Ukraine
14/16, Drahomanov St. Lviv, Ukraine, 79005
3 – Lviv National Polytechnic University, Ukraine
12, Bander Str, Lviv Ukraine, 79013
4 – Danylo Halytsky Lviv National Medical University, Ukraine
69, Pekarska St., Lviv, Ukraine, 79010

dr_r_lesyk@org.lviv.net; stoika@cellbiol.lviv.ua

Novel oligoelectrolytes of block, comb-like and branched structure containing hydrophilic backbone with hydrophobic domains and side branches of controlled length and functionality were designed and synthesized at Lviv National Polytechnic University. Their properties allow easy immobilization of various compounds including completely water insoluble ones. The main goal of this work was to investigate if novel oligoelectrolyte polymeric complexes can affect cytotoxic action of anticancer drugs towards tumor cell lines. We compared such action of doxorubicin (Dx) with the action of novel 4-thiazolidone compound Les-3120 recently synthesized at Danylo Halytsky Lviv National Medical University. Human Jurkat and CCRF-CEM T-cell leukemia cells and murine L1210 leukemia cells were used as targets. Antineoplastic activity of Dx-polymeric complexes was significantly higher than such activity of free Dx. The highest effect of these complexes was found at low concentrations of Dx (0.1–0.2 ug/ml), while there were no differences in cytotoxic action of free Dx and Dx-polymer complexes at high concentrations of Dx (1-2 ug/ml). This conclusion was confirmed by Western blot analysis of the cleaved forms of caspase-3, caspase-7, as well as their substrates PARP-1 and DFF45 in targeted Jurkat T cells. DAPI staining of human breast adenocarcinoma MDA-MD-231 cells also showed that Dx-nanocarrier complexes induced more intensive DNA fragmentation comparing with free Dx.

Although novel 4-thiazolidone compound Les-3120 possesses growth-inhibiting action towards various mammalian tumor cell lines, its anticancer potential is substantially restricted by its poor solubility in water media. We demonstrated that application of olygoelectrolyte carriers resulted in creation of water-soluble forms of this drug with the same cytotoxic activity, while additional functionalization of Les-3120-nanocarrier complexes with polyethylene glycol (PEG) increased 5 times their antineoplastic potential comparing with Les-3120 dissolved in DMSO. This effect can be explained by high lipophilic activity of PEG-functionalized nanocarriers gaining a capacity of these drug-nanocarrier complexes to targeted action towards plasma membranes and organelle (ex. mitochondria) membranes.

This work was partially supported by STCU grant № 4953.

Characteristics of colorectal cancer operated at University Hospital Split, Croatia

Mihanovic Jakov, Tomic Snjezana

Department of Pathology and Department of Surgery, University Hospital Centre Split, Croatia
Mosorska 4, 21251 Zrnovnica, Split, Croatia
mihanovic@gmail.com

Background. Colorectal cancer is the most common malignant disease of digestive tract. The main risk factors are age over 50, obesity, sedentary lifestyle, low-fiber diet and high-fat food intake. Clinical presentation includes blood stained stool, changes in bowel habits and often-obstructive symptoms. Almost 98% of large bowel malignant tumors are adenocarcinomas. Radical surgery is preferable treatment. The prognosis is most closely related to the stage of the tumor at the time of diagnosis. Dukes A stage is 100% curable but survival dramatically drops in stage Dukes C to only 23 %, and in presence of distant metastasis (Dukes D) to less than 5%. To increase number of cured patients, it is necessary to improve early detection of colorectal cancer. Implementation of screening programs such as occult blood test is recommended.

Objectives. The aims of this study were to determine characteristics of colorectal cancer operated in University Hospital Split during 3-year period.

Methods. Surgically removed colorectal specimens during 3-year period were analyzed and the following patient data were obtained: sex, age, cancer location and histological stage. Only adenocarcinomas were analyzed.

Results. A total number of patients operated because of colorectal adenocarcinoma were 576. There were significantly more men (N=345, 60%) than women (N=231, 40 %) ($p < 0.001$). Most of the patients (89%) were over 50. No one was under 30, and there were 10 patients over 85. Tumor was located on rectum or rectosigmoid junction in 291 patients (53%), and on the rest of the colon (caecum, ascending, transverse, descending colon and sigmoid) in 261 patients (47%) without significant difference regarding sex distribution ($p = 0.275$). Stage of the tumour in the majority of patients was Dukes B (N=234, 43%), followed by Dukes C (N=177, 33%) and Dukes D (N=78, 14%). Only 55 (10%) patients were operated in Dukes A stage. Significant age and sex distribution differences were not found between patients with different tumor stages.

Conclusion. Only 10% of patients were diagnosed with the earliest stage of the colorectal cancer. Many patients presented in advanced stage, when surgery is of limited value and recovery is uncertain. It is necessary to increase percentage of patients presenting in potentially curable stage. Early detection is a key and it can be accomplished by screening programs for population over 50, which are at greatest risk for colorectal cancer. Results of this study put us in east-European transitional countries average. Further efforts are necessary to improve population education in balanced diet, regular exercise and early cancer symptoms.

Characterization of novel mTOR-kinase splicing isoforms

[1]*Skorokhod Oleksandr* , [1]*Nemazanyy Ivan,* [1]*Panasyuk Ganna,* [1]*Filonenko Valeriy* [1,2]*Zhyvoloup Oleksandr and* [1,2]*Gout Ivan*

[1]Department of Cellular Signaling, Institute of Molecular Biology and Genetics, Kyiv, Ukraine. 150, Zabolotnogo St., Kyiv, Ukraine, 03680

[2]University Collage Londone, Gower Street London WC1E 6BT, UK.

fiwinner@ukr.net: i.gout@biochemistry.ucl.ac.uk

Background. The mammalian target of rapamycin (mTOR) is a conserved serine/threonine kinase, which regulates cell growth, survival and metabolism in response to environmental signals affecting protein synthesis and cytoskeleton dynamics. Deregulation of mTOR-coordinated signaling has been associated with various human pathologies, including diabetes, inflammation and cancer. mTOR kinase forms a multisubunit complex with numerous protein partners. In mammalian cells two distinct complexes have been identified: mTORC1 in which mTOR is bound to the protein partner raptor and mTORC2 in which mTOR is bound to another protein partner called rictor. These protein complexes appear to have distinct biological functions.

Recently, using RT-PCR analysis of various human cell lines our research group has identified the existence of potential mTOR splice variants. Among them – TOR-β isoform, have been characterized as a potential oncogene.

Objectives. The aim of our current studies was to prove the existence of additional mTOR isoforms - TORγ and TORε on RNA and protein levels in mammalian cells.

Results. The bioinformatical analysis of TORγ and TORε primary structure allowed determining the absence of several functional domains in their structure compared to a full-length TORα molecule. Both isoforms TOR-γ and TOR-ε were cloned in eukaryotic vector pcDNA3.1 and stable cell lines were obtained.

Also, a fragment of C-terminal part of mTOR was cloned, overexpressed and purified from bacteria cells. This recombinant protein was used for rabbit immunization and TOR-specific polyclonal antibodies generation. The application of generated antibodies allow to detect the presence in some mammalian cell lines and tissues not only TORα (290kDa), but also several bands of proteins with lower molecular weight, similar to TORβ, TORγ and TORε isoforms.

The investigation of phosphorylation status of up-stream and down-stream effectors of mTOR (Akt, S6K1, S6 protein) in stable cell lines overexpressing TORγ and TORε had showed that there status was differed compere to wild type cells. Also, we detected that phosphorylation status of TORγ, like TORα, is rapamycine sensitive, in contrast to TORε isoform. Finally, analysis of oncogenic properties of TORε and TORγ by soft agar colonies formation assay had demonstrated that overexpression of TORε, but not TORγ, lead to 1.5 fold increase in colonies number.

Conclusion. The presented data demonstrate the existence of potential isoforms of mTOR (TORγ, TORε) with possible oncogenic potential of at least for TORε.

Deep proteome characterization as a tool for identification of novel intraamniotic infection and inflammation biomarkers in preterm birth patients

[1]Tambor Vojtech, [1]Lenco Juraj, [2]Menon Ramkumar, [3]Kacerovsky Marian, [1]Link Marek and [1]Stulik Jiri

1 Institute of Molecular Pathology, University of Defence, Hradec Kralove, Czech Republic
2 Perinatal Research Center, Women´s Hospital, Centennial Medical Center, Nashville, TN, USA
3 Department of Obstetrics and Gynecology, Charles University in Prague, Faculty of Medicine Hradec Kralove, University Hospital Hradec Kralove, Czech Republic

tambor@pmfhk.cz

Background: Intraamniotic infection and inflammation (IAI) has been demonstrated to be associated with a significantly increased rate of neonatal adverse outcome, even in the absence of demonstrable positive cultures. The biochemical and protein composition of amniotic fluid is altered during pregnancy and reflects both physiological as well as pathological changes in the fetomaternal compartment. Modern proteomic technologies are able to detect and characterize even the slightest changes in protein composition of various biological matrices. Thanks to the ability to both identify and quantify a large number of proteins, this approach seems to be a very promising one for the detection of changes in amniotic fluid protein composition and for the identification of possible biomarkers for the prediction pregnancy related complication.
Objectives: We employed advanced proteomics in identification of novel potential biomarkers of IAI. The study was performed on amniotic fluid samples from preterm birth patients with confirmed (*n=31*) and ruled out (*n=26*) IAI.
Results: We successfully identified and quantified 847 amniotic fluid proteins (5% FDR) and selected more than 50 candidates, which showed dysregulated abundance between the two patient groups. To illustrate, these include neutrophil defensin 3, a range of histone proteins (H2, H3, H4 etc.), antileukoproteinase and other proteins known to be involved in host against pathogen response, tissue remodeling and cellular death.
Conclusion: We used multidimensional shotgun proteomics to describe the amniotic fluid proteome and characterize differences among individual patients group, which resulted in a rich group of novel biomarker candidates. These are currently being validated using complementary methods, both antibody-based techniques as well as targeted proteomics approaches.

Translational Research and Nano – Biotechnology Young Scientist Poster Presentation

Expression of T-type calcium channels during genesis of absence epilepsy in WAG/Rij rats

Batiuk M.Y., Boldyryev O.I. Shuba Y.M.
International Center of Molecular Physiology, NAS of Ukraine
4, Bogomoletz Str., Kyiv, Ukraine 01024
myhaelis@gmail.com

Background. Childhood absence epilepsy (CAE) is a non-convulsive form of epilepsy with incidence nearly 2-8 per 100000 children. It is characterized by impaired consciousness and SWD activity on EEG. Meeren et al. (2002) revealed "cortical focus" of SWD in somatosensory cortex of the upper lip (S1ULp zone of somatosensory cortex). Some investigators showed involvement of T-type channels SNPs in disease susceptibility (Chen, 2003) and increased expression of these channels in thalamus during disease progression (Broicher, 2008). Furthermore, non-specific blocker of T-channels ethosuximide inhibits SWD activity.

The **aim** of this study was to identify links between absence epilepsy and expression of T-type channels in "cortical focus" and thalamic laterodorsal (LD) nucleus.

We used WAG/Rij rat strain as an animal model of absence epilepsy. We investigated three T-channels ($Ca_v3.1$, $Ca_v3.2$, $Ca_v3.3$) mRNA expression in laterodorsal (LD) nucleus and "cortical focus" in WAG/Rij and control Wistar rats using TaqMan Real-Time PCR system. Three age groups of rats were taken for expression measurements in "cortical focus" (10 days, 25 days, 6 months) and two groups for measurements in LD nucleus (10 and 25 days).

Results. T-channels mRNAs expression was increased in "cortical focus" of WAG/Rij rats as compared to control Wistar rats. We observed 1.8-fold increase of $Ca_v3.2$ mRNA level in 10-days old rats, 2.5-fold increase in 25-days old group and insignificant raise in 6-months old rats. We identified almost the same levels of $Ca_v3.1$ mRNA in 10-days old epileptic and control rats, 2-fold increase in 25-days old WAG/Rij rats and 2.5-fold raise in 6-months old group. There was observed 1.3-fold augmentation of $Ca_v3.3$ mRNA expression in 10-days old group of epileptic rats, 1.4-fold increase in 25-days old group and the same levels in 6-month old WAG/Rij and Wistar rats. LD nucleus levels of T-channels mRNAs were decreased in epileptic rats. The levels of $Ca_v3.3$ and $Ca_v3.2$ mRNAs were the same in 10-days old WAG/Rij and Wistar rats and decreased by 1.6 times in 25 days old epileptic rats. We observed 1.8-fold decrease of $Ca_v3.1$ mRNA level in 10-days old epileptic rats and 3-fold diminution in 25-days old rats.

Conclusion. Elevated expression of $Ca_v3.1$ and $Ca_v3.2$ in "cortical focus" after puberty may lead to hyper excitability of neurons and generation of SWD seen in epilepsy. Expression of T-type channels in LD nucleus was decreased. As neurons of LD nucleus are over activated during spike-wave discharges, lowering of T-channels expression might indicate some compensatory mechanism against such over activation. Our data also show that Cav3.1 and Cav3.3 are the main basis of T-current in LD thalamic neurons during early ontogenesis.

Follow up of viral RNA after coxsackievirus B3 oral and intraperitoneal routes of experimental infection using recombinant virus vector

[1]Baďurová Miriama,[2]Precechtelová Jana, [1]Sojka Martin, [1]Štípalová Darina, [2]Gomolčák Pavol, [1]Bopegamage Shubhada

1. Slovak Medical University,
12, Limbova 833 03 Bratislava, Slovak Republic
2.Cytopathos s.r.o.,
Limbova 5, 833 01 Bratislava, Slovak Republic
shubhada.bopegamage@szu.sk

Background: Coxsackievirus infections often display a subclinical course or may lead to only mild disease, but the outcome of the infections may be serious or even fatal. Coxsackieviruses B3 (CVB3) are more known as aetiological agents of inflammatory heart diseases and are implicated in the pathogenesis of sub-acute, acute and chronic human myocarditis or dilated cardiomyopathy

Recombinant CVB3 virus expresses an enhanced green fluorescence protein (eGFP). eGFP was selected as the optimal marker protein because it is relatively small in size enabling its incorporation into recombinant CVB and it is relatively resistant to photo bleaching. Understanding aspects of coxsackieviral diseases such as spread, transmission, the role of host immune system, the interaction between host and virus and pathogenesis will help in prevention and control of infection diseases.

Aim: To follow the spread and pathophysiological events after CVB infection using CVB3 recombinant virus during the 24 hours interval after oral and intraperitoneal (ip.) infection by studying the presence of viral RNA in the organs of the infected mice and compare with the eGFP flourescence.

Results: Organs (heart, pancreas, spleen, small intestine and brain) were collected at 2-hour intervals and studied for the presence of viral RNA by RT-PCR. In ip. infected mice viral RNA was detected in heart from 2h post infection (p.i.) in 2/3 mice, in pancreas from 2h p.i. in 1/3 mice, in spleen virus appeared between 4 - 6h p.i.in all 3 mice, small intestine from 2h p.i.in 3/3 mice and in brain from 2h p.i. in 3/3 mice.

In orally infected mice pancreases were completely negative till 12h p.i., in spleen the virus was observed from 8h p.i. in 2/3 examinated mice, in small intestine from 2 h p.i. in 1/3 mice and brain and heart showed negativity to 12h p.i mice. Uninfected controls were negative.

Conclusions: Almost in all organs of ip. infected mice the virus was detected, at 2 h p.i. Orally infected mice were more protected from virus infection. Virus was presented at interval 2 h p.i. only in small intestine. All others orally infected organs of mice showed negativity for presence of virus at this early interval. In ip. infected mice the virus spread was rapid.

Acknowledgements: We thank Prof. J. M. Galama from Nijmgen, the Netherlands for scientific discussions, Dr. A. Henke from Jena, Germany for supplying eGFP-CVB3. For financial support- MZSR code: 2007/03- RUVZBB-01 and the Norwegian financial support mechanism, Mechanism EEA and Slovak Government - Project SK0082.

Peroxisome proliferator-activated receptor-α agonist BAY PP1 attenuates renal fibrosis

[1,2,3]Boor Peter, [4]Celec Peter, [2]Martin Ina V., [2,5]Villa Luigi, [4]Hodosy Július, [1]Klenovicsová Kristína, [5]Esposito Ciro, [6]Schäfer Stefan, [6]Albrecht-Küpper Barbara, [2]Ostendorf Tammo, [7]Heidland August and [1]Šebeková Katarína

[1] Department of Clinical and Experimental Pharmacotherapy, Research Base of Slovak Medical University, Bratislava, Slovakia;
[2] Division of Nephrology
[3] Institute of Pathology, RWTH University of Aachen, Germany;
[4] Institute of Molecular Biomedicine, Comenius University, Bratislava, Slovakia;
[5] Unit of Nephrology, Dialysis and Transplantation, University of Pavia, Italy;
[6] Bayer Schering Pharma AG, Cardiology Research, Wuppertal, Germany;
[7] Department of Internal Medicine, University of Würzburg, Germany.
kata.sebekova@gmail.com

Recent studies have suggested renoprotective effects of peroxisome proliferator-activated receptor-α (PPAR-α), but its role in kidney fibrosis is unknown. We examined the effect of a novel PPAR-α agonist, BAY PP1, in two well established rat models of renal fibrosis: the unilateral ureteral obstruction (UUO) and the remnant kidney model (5/6 nephrectomy). We previously presented the preliminary data in the UUO model. Here we show further experiments confirming the antifibrotic role of PPAR-α in renal fibrosis.
In healthy animals, PPAR-α was expressed in renal tubular cells but not in interstitial cells, like e.g. fibroblasts. Upon induction of fibrosis, PPAR-α was significantly down regulated, and treatment with PPAR-α agonist BAY PP1 significantly restored its expression. In UUO, treatment with BAY PP1 significantly reduced tubulointerstitial fibrosis, proliferation of interstitial fibroblasts and TGF-β_1 expression, whereas treatment with a less potent PPAR-α agonist, fenofibrate, had no effects. Treatment with BAY PP1 in the remnant kidney model halted the decline of renal function and significantly ameliorated renal fibrosis. This is of particular importance, since the treatment was initiated in already established disease, i.e. a clinically highly relevant situation. *In vitro*, BAY PP1 had no direct effect on renal interstitial fibroblasts but reduced collagen, fibronectin and TGF-β_1 expression in tubular cells. Conditioned media of BAY PP1-treated tubular cells reduced proliferation of fibroblasts in a paracrine fashion.
In conclusion, renal fibrosis is characterized by a reduction of PPAR-α expression, and the PPAR-α agonist BAY PP1 is capable of restoring it, thereby reducing renal fibrosis. This is most likely mediated *via* affecting the cross talk between tubular cells and fibroblasts. These data suggest that potent PPAR-α agonists could be a novel treatment option in renal fibrosis.

Origin of spontaneous and induced by the chemical mutagens point mutations and enzymatic control over them

[1,2]*Brovarets O. O.*, [1,2]*Hovorun D. M.*

[1] Institute of High Technologies, Taras Shevchenko Kyiv National University,
2, Hlushkova Ave., Kyiv, 03187, Ukraine;
[2] Institute of Molecular Biology and Genetics, NAS of Ukraine,
150, Zabolotnogo Str., Kyiv, Ukraine, 03680
brovarets@list.ru

Background. For half a century, prototropic tautomerism of nucleic acid bases has been considered an origin of spontaneous point mutations arising in DNA, nevertheless the elementary physico-chemical mechanisms of its arising still remain uncertain.

Aim. To establish molecular models of the spontaneous and induced by chemical mutagens transitions and transversions being based on the physico-chemical mechanisms of the DNA bases tautomerization within Watson-Crick and wobble base pairs using quantum-chemical calculations.

Results. Physico-chemical mechanisms of the transitions, in particular incorporation and replication errors in DNA, based on the novel structural ideas in the area of the spontaneous point mutagenesis were revealed and validated.

Replication errors arise due to tautomerization of Gua·Cyt and Ade·Thy Watson-Crick base pairs to dynamically stable Gua·Cyt* and Gua*·Cyt or Ade*·Thy and Ade·Thy* mispairs respectively formed by rare tautomers (marked by an asterisk) and quasiisomorphic to wobble mispairs, and incorporation errors – due to tautomerization of Gua·Thy and Ade·Cyt wobble mispairs formed by the bases in their canonical forms to isosteric Watson-Crick, dynamically stable Gua*·Thy and Ade·Cyt* mispairs involving rare tautomers accordingly. Transition states of such conversions are planar symmetric ion pairs stabilized by H-bonds.

It was established that mutations induced by the mutagenic nucleobase analogues – halogen derivatives of uracil (5-bromouracil, 5-chlorouracil and 5-fluorouracil), 2-aminopurine and 6H,8H-3,4-dihydro-pyrimido[4,5-c][1,2]-oxazin-7-one – arise due to the decrease of activation barrier of tautomerization and the increase of the population of mispairs involving rare tautomers.

Physical model of the recognition of Watson-Crick base pairs by DNA-binding proteins from the major groove side was elaborated. There are strong grounds to suggest that asparagine and glutamine side chains of these proteins are the best-suited candidates to participate in the process due to their spatial arrangement about base pairs. This model allows the inhibition of biosynthesis of mispairs formed by both rare and canonical tautomers of DNA bases.

We have investigated all possible purine-purine mispairs containing template base only in anti-configuration. It was established that only those of them, which can easily adopt Watson-Crick, shape, cause spontaneous transversions arising.

Conclusion. Novel physico-chemical models of spontaneous and induced by chemical mutagens, namely the analogues of nucleobases, transitions and transversions were elaborated and analyzed.

Pharmacognostic evaluation of *Salix spp.* honeydew and nectar honeys and their antioxidant capacity

[1]Jerković Igor, [2]Tuberoso Carlo I. G., [2]Bifulco Ersilia and [3]Marijanović Zvonimir

[1] Dep. of Organic Chemistry, Faculty of Chemistry and Technology,
University of Split, N. Tesle 10/V, 21000 Split, Croatia

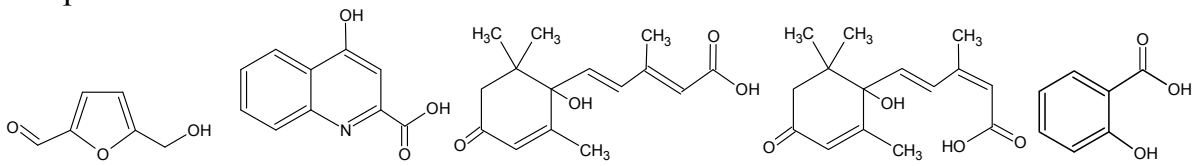
[2] Dip. Farmaco Chimico Tecnologico, University of Cagliari, via Ospedale 72, 09124 Cagliari, Italy

[3] Department of Food Technology, Marko Marulić Polytechnic in Knin,
Petra Krešimira IV 30, 22300 Knin, Croatia

igor@ktf-split.hr

Rare unifloral nectar and honeydew willow (*Salix* spp.) honeys from Croatia were investigated by direct RP-HPLC-DAD method as well as by headspace solid-phase micro extraction (HS-SPME) and ultrasonic solvent extraction (USE) followed by GC and GC-MS analyses in order to identify and quantify compounds that can be used as possible markers of their origin. It is now recognize that many phytochemical compounds can have health-promoting activities and a honey is known to be rich in both enzymatic and non-enzymatic antioxidants.

HMF (**1**), diastase activity and CIE L*a*b*C*h* chromatic coordinates of all the samples were evaluated. Abscisic acids (ABA) are typical of willow nectar honey, with a predominance of *cis*, *trans*-ABA (**2**) on *trans*, *trans*-ABA (**3**) [98.2 and 31.7 mg/kg, respectively]. Kinurenic acid (**4**) and salicylic acid (**5**) are useful to mark willow honeydew honey. The proposed HPLC-DAD method proved to be easy and reliable to identify the two different *Salix* spp. honeys, being not affected from any sample preparation artifact. The use of HS-SPME and USE had advantageous results over the use of one single technique, as it provided different complementary chromatographic profiles for a comprehensive screening of the honeydew volatile composition. *Salix* spp. nectar and honeydew honeys proved to be two completely different honeys because, besides color attributes, they show different antioxidant properties and specific compounds.



1 **2** **3** **4** **5**

Antioxidant and antiradical activities of willow honeys were evaluated using FRAP and DPPH tests, respectively. Total antioxidant activity measured with the FRAP assay ranged from 3.2 to 12.6 mmol $Fe^{2+/}$kg, and antiradical activity measured with the DPPH assay ranged from 0.6 to 3.0 mmol TEAC/kg in nectar and honeydew honeys, respectively.

Non – viral nanoscale-based delivery of antisense oligonucleotides enhances inhibition of the cellular prion expression *in vivo*.

[1]Ivanytska L., [2]Stadnyk V., [1]Kozak M., [3]Zaichenko A., [3]Mitina N., [1]Vlizlo V.
[1]Institute of Animal Biology NAAS, Lviv, Ukraine,
38, Stusa str., Lviv, Ukraine
[2] Eötvös Loránd University ,
Egyetem tér 1-3. Budapest, 1053 Hungary
[3]Lviv Polytechnik National University, Ukraine
12, Bandery Str, Lviv, Ukraine 79013
ivanytska.lyudmyla@gmail.com

Neurotoxicity from accumulation of misfolded cellular prion (PrP^C) is thought to drive pathogenesis in transmissible spongiform encephalopathies (TSE). There is no cure for these disorders. It was ascertained that presence of normal PrP^C is absolutely necessary for illness development. Targeting PrP^C has the potential to remove substrate for the TSE pathogenesis so this is a promising strategy for development of means for treatment and prevention prion infections. In accordance to this the aim of our study was to decrease the cellular prion expression level *in vivo* by antisense oligodeoxyribonucleotides (asODNs) toward prion mRNA.

But instability of asODNs in blood serum and obstacles of biological barriers demand effective delivery system for *in vivo* application of the antisense technology. So first novel telechelic poly dimethylaminoethylmethacrylate (polyDMAEM) oligoelectrolite (Mw = 5000 g/mol) with end ditertiary peroxide fragment was developed. DNA – binding ability was checked by UV-spectroscopy and turbidimetry.

For effective inhibition of PrP^C gene mRNA translation three asODNs were designed. On 24^{th} h. of incubation L1210 cells with complex asODN and polyDMAEM all three asODNs showed 95-98% ($r<0.01$) efficiency in decreasing PrP^C expression. Western blot analysis and immunohistochemistry demonstrate inhibition of PrP^C expression in the spleen of Wistar rats by almost 90% ($r<0.05$) on 48^{th} h post i. v. injection of asODNs-polyDMAEM complex. It is observed 80% decrease of PrP^C content in the intestine. Investigated asODN nanoscale delivery system demonstrates most significant results in rat brain. Thus asODN immobilized on poliDMAEM was capable to overcome the blood-brain barrier and decrease PrP^C expression to 60% from a control level ($r<0.01$).

Testing of polyDMAEM *in vitro* on cell line L1210 and primary rat fibroblast cells showed very low toxicity of polymer. After single i.v. injection of polyDMAEM to rat's physical activity and other external manifestations of animal behavior were not changed. Decrease of feed and water consumption was not observed among all experimental animals. Results of total protein concentration, activities of marker ferments in serum and histology investigation of liver, kidneys, spleen, intestine and brain indicate certain metabolic stress of liver and intracerebral capillary permeability violation on 48 h after administration of tested compounds.

Our investigations show that polyDMAEM has a high potential for use in CNS delivery systems. As a result of carried out research there were developed effective asODNs and their delivery system for inhibition of prion mRNA translation.

^{1}H NMR spectroscopy study on the impact of MRPs-rich diet on urine metabolome in healthy rats

[1]Kollárová Radana, [2]Pronayová Nadežda, [3]Boor Peter, [1]Šebeková Katarína

[1]Department of Clinical and Experimental Pharmacotherapy, Slovak Medical University, Bratislava, [2]Department of NMR Spectroscopy, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, [3]Institute of Pathology and Department of Nephrology, RWTH University, Aachen, Germany

kata.sebekova@gmail.com

Non-enzymatic glycation of proteins is a complex series of parallel and sequential reactions, collectively called the Maillard reaction, occurring during thermal processing of food. Maillard reaction products (MRPs) are formed via spontaneous reaction of free amino-groups of proteins and reactive sugars, and render baked and fried food its characteristic color, odor and taste. Some MRPs are biologically active substances. In vivo formed analogues of MRPs are called advanced glycation end products (AGEs). Exaggerate accumulation of AGEs in disease states (e.g. diabetes, chronic renal failure) alters the structure and function of proteins and elicits negative heath effects via interaction of AGEs with their specific cell surface receptors. It is accepted that, at least a part of alimentary MRPs is absorbed into circulation, where they may potentiate the toxic effects of AGEs. The aim of the study was to investigate the impact of dietary MRPs on urine metabolome, with regards to presence/absence of MRPs-derived aroma substances.

MRPs-rich diet was prepared by replacing 25% (wt/wt) of standard rat chow by bread crusts. Aroma extracted MRPs-rich diet contained 25% of aroma-extracted bread crusts (obtained via quadruple extraction of bread crust with diethyl ether). 24 male Wistar rats (180-220g) were randomized into 3 groups (n=8, each) and fed for 3 weeks either by standard rat chow (controls), MRPs-rich- or aroma extracted MRPs-rich-diet. 24h urine collection was performed before, and weekly during the experiment. Urine samples were analyzed using ^{1}H NMR spectroscopy. Obtained data were evaluated using multivariate statistic analysis.

The composition of urine of the animals on standard rat chow differed from that of the rats on both MRPs-rich diets. However, at week 1 and 2 metabolomes of aroma-rich and aroma-extracted diets consuming rats did not differ significantly. The impact of aroma substances appeared only in samples collected on the last day of the experiment: an indication of separation between the groups was observed.

It is concluded that consumption of MRPs-rich diet alters urine metabolome. According to our best knowledge this is the first study dealing with the impact of MRPs-rich diet on urine metabolome. Identification of single metabolites contributing to between group differences requires further analyses.

Study was supported by SMU internal grant No.199005 (KK) and CENDO grant No.194001(KŠ).

Evaluation of osteocyte dedifferentiation process

[1,4]Pejda S., [2]Kizivat2 T., [3]Fatahi M., [3]Igwe J., [4] Horowitz M. C., [3]Maye P., [3]Rowe D. W., [3]Kalajzic I.

[1]School of Medicine, University of Split, Croatia,
[2]School of Medicine University of Osijek, Croatia,
[3]University of Connecticut Health Center, Connecticut, USA,
[4]Yale University, New Heaven, Connecticut USA.

spejda@gmail.com

Background: Dedifferentiation is the progression of cells from a more differentiated to a less differentiated state. Presently, there is no evidence for the ability of mature osteoblast lineage cells to dedifferentiate. However, if possible, de-differentiation of mature osteoblast lineage cells could provide an additional source of osteoblast population capable of proliferation and differentiation. This could become an important anabolic strategy for older individuals, where a decrease in the osteoprogenitor pool may contribute to decreased bone formation. **Aim**: Our aim is to investigate if preosteocytes and osteocytes under the conditions of high demand can undergo a de-differentiation, accompanied by dramatic changes in gene expression. This process would generate cells that could exhibit proliferation potential, followed by differentiation into bone matrix producing osteoblasts. **Methods and Results**: To determine whether some of the outgrowing cells were derived from dedifferentiating osteocytes, we used transgene lineage markers (DMP1GFP and DMP1Cre). Cell cultures were made from bone chips using DMP1GFP mice. As the DMP1 is active during an osteocyte stage, we did not observe GFP expression in cells that grew out of the bone chips. This indicates that these cells do not express this osteocyte-specific promoter. However, this does not preclude the possibility that BCOC (bone chip outgrown culture) are derived from osteocytes. To evaluate whether the BCOC are derived from cells that once were osteocytes, we have established bone chip cultures from a breeding derived from DMP1Cre x Rosa26R or Ai9 reporter strain in which the GFP-Tomato is expressed only in cells in which Cre is currently active or was active at some point during differentiation. In contrast to the DMP1GFP cultures, we observed lacZ or GFP-Tomato in a high proportion of cells that grew out of the chips just 3-5 days after the initiation of culture. As we have demonstrated that BCOC's do not have DMP1 promoter active at that stage in culture (DMP1GFP negative), these data indicate that the Tomato expression is due to the historical activation of reporter construct by DMP1Cre. Thus, the cells that are Tomato+ were at some point DMP1Cre+. These indicate that osteocytes can crawl out bone chips and dedifferentiate in vitro. The BCOC express some of the genes characteristic of osteoblast/osteocytes and following transplantation their fate is to become embedded into bone matrix. **Conclusion**: Utilizing a transgenic model we evaluated the ability of de-differentiated osteocytes to repopulate the osteoblast niche.

Single nucleotide polymorphisms in obesity-related genes

Sliz I, Gogalova V, Smolkova B, Dusinska M, Gasparovic J, Raslova K, Horska A, Dzupinkova Z, Staruchova M, Volkovova K.

Department of Experimental and Applied Genetics, Slovak Medical University, Limbova 14, 833 03, Bratislava, Slovakia

sliz.ivan@gmail.com

Background: Numerous connections between chronic metabolic disorders, including obesity, and cardiovascular diseases (CVD) have been established and are currently well recognized. Obesity, which represents one of many different cardiovascular risk factors, exerts effects predominantly on the heart and circulation, on both directly and also by its association with other known risk factors such as hypertension, dyslipidemia or insulin resistance. These effects tend to be more prevailing with increased weight gain, especially in younger subjects even prior becoming obese.
Objective: The aim of this study was to investigate the association between the genotype, environmental factors and potential health risks resulting from both obesity and CVD in a group consisting of 2403 randomly selected subjects (1001 men, 1402 women) who were approximately the same age (40.5 ± 0.7 years) and came from 7 different Slovak regions. Several following polymorphisms (SNP) primarily contributing to obesity were genotyped: growth hormone secretagogue receptor (*GHSR*; rs495225 and rs572169), beta-1 adrenergic receptor (*ADRB1*; rs1801252), beta-2 adrenergic receptor (*ADRB2*; rs1042713 and rs1042714), 5-hydroxytryptamine receptor 2C (*HTR2C*; rs6318), leptin receptor (*LEPR*; rs1137100) and alpha-ketoglutarate-dependent dioxygenase FTO (*FTO*; rs9939609). The frequencies of these variants were analyzed in relation to anthropometric characteristics like body mass index (BMI) and waist circumference (WC), a wide range of biochemical parameters such as serum total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), non-HDL cholesterol (non HDL-C), total to HDL-C ratio (TC/HDL), triglycerides (Tg) and glucose (Glu) levels, as well as to volunteers' personal and family anamneses and other lifestyle data.
Results: Homozygous carriers of variant allele rs1137100 of the *LEPR* gene had significantly higher BMI ($P = 0.005$) and WC ($P = 0.001$) compared to heterozygotes combined with reference group. Among the other polymorphisms tested in this study, a significant association was observed between the homozygous variant genotype of the *FTO* gene (rs9939609) and increased blood Glu levels ($P = 0.008$).
Conclusion: These findings lead us to the suggestion that the rs1137100 variation in the leptin receptor gene contributes to weight gain and variability in body fat distribution. The observed presence of the *FTO* rs9939609 variant associated with increased Glu level, which belongs to the symptoms of the metabolic syndrome, may support the hypothesis that variantions in the FTO locus are involved in increasing risk of developing CVD.

Differences in pathogenesis of coxsackievirus B4 isolates of different origin in a mouse model

Sojka Martin, Precechtelova Jana, Badurova Miriam, Stipalova Darina, Bopegamage Shubhada
Slovak Medical University, Bratislava
Limbova 12, 833 03 Bratislava, Slovak Republic.
shubhada.bopegamage@szu.sk

Background: Coxsackieviruses (CV) are ubiquitous faecal-orally transmitted, small RNA viruses, belonging to the family *Picornaviridae*. HEV are now classified into four species: human enterovirus A-D. These viruses cause a wide variety of diseases and it is extensively known that non-polio enteroviruses are the most common cause of aseptic meningitis in adults and in children as well. The presence of mouse coxsackie-adenovirus (mCAR) receptor, a corresponding receptor to human coxsackie-adenovirus (hCAR), one of the receptors used by virus in process of target cell entry, has made mouse the most common experimental model for studying of coxsackieviral infection. Experimental mouse models are used to study the enterovirus (mainly coxsackievirus) infection.
Objectives: The aim was to study and compare the viral pathogenesis, organ and tissue tropism of coxsackievirus B4 isolates of different origin.
Results: In the study, CVB 4 isolates of human origin (cerebrospinal fluid and stool) and isolate from treated wastewater were compared. CD1 mice were infected orally by model viruses (previously plaque purified) using oral gavage with a defined infectious dose. Presence of viral RNA in heart, pancreas, brain, small intestine, blood and stool was observed by means of enterovirus-specific PCR. Histopathological changes of infected tissues were studied. Significant differences in the presence of virus were found between viruses of different origin. In CSF isolate infected mice, viraemia occurred at day 10 post infection (p.i.), while in stool and environmental isolate infected mice viraemia was present at day 5 p.i. At day 5 and 10 p.i. viruses were present in mice organs, but with differences depending on the origin of virus. Stool and environmental isolate showed only cardiotropic and pancreatotropic properties, unlike CSF isolate detected in brains as well. At day 45 post infection, the virus was detected only in brains of mice infected with CSF isolate. No significant histopathological changes were observed in infected tissues.
Conclusion: Significant differences in organ tropism of coxsackieviruses of different origin were observed. The *in vivo* model infection is of importance for enterovirus infection study on tissue, organ and host level. A thorough study of model infection and treatment is the only way how to move forward in our knowledge about enterovirus pathogenesis, mode of virus adaptation to different tissues and organ tropism.
Acknowledgements: Dr. Z.Sobotova, Bratislava Slovak Republic (for the samples), Financial support- MZSR code: 2007/03- RUVZBB-01 and the Norwegian financial support mechanism, Mechanism EEA and Slovak Government - Project SK0082.

Persistence of viral RNA in experimental infection of coxsackievirus B5

Stipalova D., Sojka M., Borsanyiova M., Badurova M., Marosova L., and Bopegamage S.

Department of Virology, Slovak Medical University,
Limbova 12, 833 03 Bratislava, Slovak Republic.

darina.stipalova@szu.sk

Background: Coxsackie B virus (CVB5) is associated often with meningitis sporadic cases of neurological diseases, and chronic diseases such as cardiomyopathy and diabetes. Molecular epidemiology of these viruses and its relation to the viral pathogenesis is important. These viruses exist as circulating heterogeneous populations of genetic variants as other enteroviruses.
Aim: The aim of our study was to follow the persistence of viral RNA in different organs of mice infected with CVB5 strains. These were mainly patient's isolate and an isolate identified as CVB5 from treated sewage sample (from the area of domicile of the patient) and the prototype strain in an outbred experimental murine model.
Results: CD-1 mice were infected with CVB5 strain Faulkner, (prototype) CVB5 – isolate from treated sewage waste and isolate from patient´s stool sample identified as CVB5. The viral RNA was detected by RT-PCR using enterovirus primers specific for the non-coding 5' region. We observed presence of RNA in the brain and heart of mice infected with the patient's isolate at day 45-post infection (p.i.).
Conclusion: We concluded that CVB5 persists in the brain and heart after oral infection of CD1 mice. The relevance of viral persistence maybe related viral origin and the genetics.
Acknowledgements: Dr. Z.Sobotova, Bratislava Slovak Republic (for the samples), Norwegian financial support mechnism, Mechanism EEA and Slovak Government - Project SK0082

Determination of excitatory amino acids in brain microdialysates by capillary electrophoresis-laser induced fluorescence detection

[1]Tábi T., [1]Wagner Zs., [2]Zachar G., [2]Csillag A., [1]Szökő É.

Semmelweis University,
Nagyvárad tér 4., 1089 Budapest, Hungary
[1]Department of Pharmacodynamics,
[2]Department of Anatomy, Histology and Embryology,

tamas.tabi@net.sote.hu

Excitatory amino acid neurotransmitters are important in various physiological and pathological brain processes. Glutamate has mainly gained attention, while involvement of aspartate in these functions is less well understood. The aim of our studies is to simultaneously follow the change of aspartate and glutamate concentration in the extracellular space, analyzing micro dialysis samples from substantia nigra of the experimental animals. An appropriate analytical method first has been developed.

Difficulties in analyzing biological samples are the limited amount of sample specimens, low analyte concentration, complex sample matrix, etc. Capillary electrophoresis (CE) with laser induced fluorescence (LIF) detection is usually used when few microL micro dialysates are to be analyzed. LIF is regarded to provide selective and sensitive detection. However, as majority of analytes has no intrinsic fluorescence, derivatization is required, considerably limiting the expected advantages. Selectivity is rather impaired, since biofluids contain lots of compounds labeled alongside with the analytes of interest. Interference also derives from the labeling reagent, and the concentration of the interfering compounds usually highly exceeds that of the analytes. The impaired selectivity is accompanied by impaired sensitivity. Furthermore, complex samples require carefully designed separation conditions to ensure appropriate resolution. Several buffer additives are commonly used simultaneously to improve selectivity and widen separation time window. The high sensitivity of LIF is also limited by the unreliable derivatization reaction at low sample concentration. Labeling at submicromolar concentrations suffers from the slower reaction rate and the increased competition with hydrolysis reaction of the derivatizing reagent. The consequence of the incomplete derivatization is the loss of linear correlation between concentration and peak area or height.

Three labeling reagents have been compared in terms of accuracy and complexity of separation conditions. In case of all labeling tags, quantitative determination can be performed down to 100 nM sample concentration. Considering other circumstances, FITC labeling was used in our further studies. Applying potassium to release transmitters, about 3 to 4 times increase of the extracellular glutamate concentration and even higher increase of aspartate has been detected. Even mild stress of animals stimulated transmitter release, the concentration about doubled in the micro dialysates. Based on these results, our method is appropriate to follow the change of neurotransmitter levels in the brain.

Gangliosides involvement in apoptotic process

Tomin Andriy, Shkandina Tanya, Bilyy Rostyslav

Institute of Cell Biology, NAS of Ukraine
14/16, Drahomanov St. Lviv, Ukraine, 79005

amtomyn@gmail.com

In our previous research it was shown, that some of human sialidases are activated during the apoptosis via caspase-involved process. Their main targets are terminal residues of sialic acid, located on glycotops of plasma membrane glycoproteins. We proved this process to be important for effective recognition and elimination of apoptotic cells by phagocytes. Also, one of sialidases, which may be activated during the apoptosis, named Neu3, is known, as "ganglioside sialidase" for it action towards sialic acid residues of membrane sialoglycosphingolipids – gangliosides. Gangliosides play significant role in intercellular communication, and it was presumed that they take part in apoptotic events. Also, gangliosides are involved in pathology of many diseases, as Guillain-Barré syndrome and lupus erythematosus, where anti-ganglioside antibodies could be produced, and autoimmune disorders are caused by failure of apoptotic blebs clearance.

We studied changes, which appear in splingolipid profile of the cell undergoing apoptosis. Gangliosides were isolated from granulocytes, viable and apoptotic (aged for 24 hours), as described in (Svennerholm, 1964), and analysed by thin-layer chromatography with different solvent systems (chloroform: methanol: water 65:35:8 and chloroform: methanol: $CaCl_2$ aqueous for general analysis and propanol: 2M ammonia 7:3 for polysialic gangliosides). Also, sphingolipid samples were digested by ceramide glycanase (EC 3.2.1.123) from *Hirudo officinalis*, glycan parts were marked with anthranilic acid (Sigma), and studied by HPLC analysis.

We revealed some increase of GM2, GM1 and globoside Gb3 in apoptotic cells simultaneously with decrease of GM3 and Gb4 globoside, which may be caused by activation of N-acetylgalactosamine-specific glycosidase. Also, the recently described abzyme (Bilyy, 2010) with sialidase activity, obtained from multiple myeloma patient, is able to cleave sialic acid from GD3 ganglioside, which may be useful in understanding of pathology of this disease.

Research of europium oxide nanoparticle toxic influence on SPEV cell culture

Pavlovich E.V., Goncharuk E.I., [1]Ganina I.I.

Institute for Problems of Cryobiology and Cryomedicine NAS of Ukraine,
23, Pereyaslavskaya str., Kharkiv, Ukraine, 61015
1Institute of Scintillation Materials of the State Scientific Institution "Institute for Single Crystals" of NAS of Ukraine,
60, Lenin Avenue, Kharkiv, Ukraine, 61072

lenapavlovich@gmail.com

To understand the influence of nanotechnology on an environment is the study of nanoparticles interplay at the model of biological objects, such as cell cultures is relevant. The aim of our investigation was study of the interaction of nano-sized compounds with cultured cells, by means of morpho-functional state of cells with incorporated NPs investigation. Effect of NPs at the viability and property to adhesion of SPEV cells were researched.

Europium oxide nanoparticles (NPs) colloid solution by the «hot solvent» method and microwave heating were synthesized. For experiments was used NPs without and with ultrasonic disaggregation.

Assessment of the NPs distribution in cultured porcine kidney epithelium transplantable line (SPEV) cells was carried out by using a scanning confocal microscope (Carl Zeiss) at 405 nm excitation. While studying of NPs penetration characteristics on SPEV cells it was found that investigated NPs luminesced in emission 540-647 nm and were localized in the cytoplasm and not penetrated in the nucleus.

In the process of estimation of toxic effects in NPs concentrations of 6.8-340 μg/ml on the viability and adhesive properties of cells it was shown that NPs in concentrations of 6.8 μg/ml did not affect on the viability of cell SPEV. It was found that NPs before and after disaggregation in 34 and 340 μg/ml concentrations decreased index of cell viability. After preliminary disaggregation NPs their toxic effects was amplified in the concentrations NPs 340 mg/ml.

It was shown that washing from NPs are not significantly reduced the toxic effect and suggested that penetration of NPs occurs in the first minutes of incubation.

Cell suspensions with internalized NPs in the growing medium were incubated, the adhesive properties of cells was studied during 1-24 hours in the inverted microscope visually. The ability of cells to adhesion decreases with growing concentration of internalized NPs. At low concentrations of europium oxide, 17 and 34 mg/ml adhesive properties of cells are preserved, but these processes proceed more slowly, which subsequently leads to slow the division. It was shown that NPs concentration 340 μg/ml irreversibly affect at the processes of adhesion and leading to cell death. Results can be a basis for prognostic test cultured cells, which has prospects of the assessment of potential harm nano-sized particles for human health and the environment.

Investigation on lanthanides doped β-$NaGdF_4$ nanocrystals - synthesis and optical characterization

Sojka B., Banski M., Podhorodecki A. , Misiewicz J., [1]Afzaal M., [2]O'Brien P.

Institute of Physics, Wroclaw University of Technology,
Wybrzeze Wyspianskiego 27, 50-370 Wroclaw, Poland
[1]Center of Research Excellence in Renewable Energy, King Fahd University of Petroleum and Minerals, Dhahran, 31261 Saudi Arabia
[2]The Manchester Materials Science Centre and Department of Chemistry, The University of Manchester, Oxford Road, Manchester, M13 9PL, UK

artur.p.podhorodecki@pwr.wroc.pl

Multifunctional bio-markers are recently of significant interest due to their huge potential in bio-medicine applications. Lanthanide doped fluoride nanocrystals (ncs) have already presented an efficient photoluminescence from variety doped ions giving visible down-converting (e.g. Eu3+, Tb3+) and infrared up-converting (e.g. Yb3+-Tm3+) emission. Gd substitution received particular attention due to magnetic properties of this ion. We propose to extend the functionality of ncs by co-doping of hexagonal NaGdF4 ncs with Eu3+ and Tb3+ ions simultaneously.

The co-thermolysis of metal trifluoroacetates was used to synthesize Eu3+ and Tb3+ co-doped β-NaYF4 and β-NaGdF4 nanocrystals. According to TEM and XRD the nanodimesional samples were highly crystallized and of the desired hexagonal phase with uniform maximum diameter sizes (~9+1 nm). The photoluminescence (PL), PL excitation and time-resolved PL were applied to investigate the properties of tunable emission spectra. Rich sets of emission bands related to f-f transitions of Tb3+ and Eu3+ ions were obtained for both samples. Due to the excitation energy migration and the cross-relaxation the emission form Tb3+ and Eu3+ can be excited simultaneously or separately, depends on the excitation energy. The conducted study suggests a strongly enhancement of the energy migration in β-NaGdF4 matrix resulting in intensive PL from Eu3+ ions.

The multicolour luminescence of bi-functional β-NaGdF4:Eu3+-Tb3+ nanocrystals opens a bright future for this material as a bioprobe.

Preparation of spr immunosensor chip for fibrinogen and soluble fibrin determination in human plasma

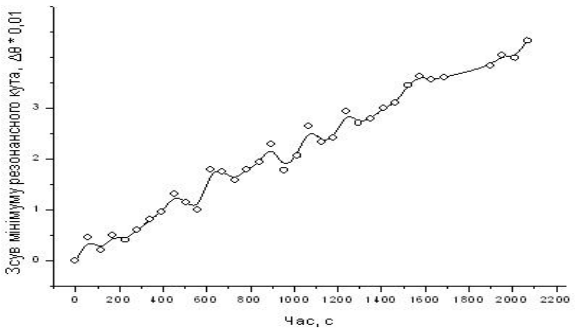
Storozhylova N. S., Bereznytsky G. K., Makogonenko E. M., Lugovskoi E. V.

O.V.Palladin Institute of Biochemistry NASU,
9, Leontovycha Str., Kyiv, Ukraine, 01601

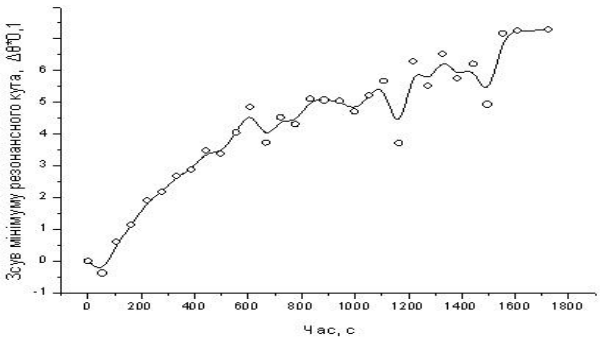
clearangel@mail.ru

Surface plasmon resonance (SPR) technology is widely used in scientific researches for investigation of biomolecules interactions in real-time and in medicine for clinical diagnostics. It provides quantitative information on specificity, concentration and activity of molecules-analytes, identification of binding targets in biologic fluids, etc. Quickness and high sensitivity are two important advantages of SPR that allows the quantification of important proteins in human plasma in 30 minutes [1].

The aim of work was preparation of the biosensor chips with antibodies covalently immobilized on the chip to quantify of protein markers in plasma of the patients with cardio-vascular diseases. We used the self-assembled monolayer (SAM), formed on the gold-plated glass, as a basis for binding of sensor molecules to the chip [1, 2]. SAMs were prepared from thiols with different length of carbohydrogenic chains – 11-mercaptoundecanoic acid (MUA) and 6-mercapto-1-hexanol (MH). We investigated binding properties of chips in dependence on MUA to MH concentration ratio. At first series of experiments SAMs for immobilization of monoclonal antibodies (monAB) was prepared at 1:4, 1:2.5, 2:3, and 1:1 MUA to MH concentration ratio. Gold-plated glass surfaces were treated by 5 mM concentration of thiols in ethanol for 7 days to form SAM. Thereafter, the COO^- groups were activated by 0.2 M carbodiimide. Anti-fibrinogen monAB II-4d were immobilized in 0.03 M phosphate buffer, pH 7.4, containing 0.3 M NaCl, at concentration 20 μg/ml. The formed chip was washed with 0.02 M HEPES, pH 7.4, containing 0.15 M NaCl, 0.005% Tween-20, and 0,005% NaN_3. The results of SPR analysis showed that chips with SAMs of MUA and MH in ratio 2:3 had much effective binding of fibrinogen (Fig). The biosensor chips for fibrin were prepared in the same way, but with anti-fibrin monAB 1-IIIc. Using SPR analysis we tested the obtained chips for Fig and fibrin binding of model solutions, prepared of purified proteins and from patients' plasmas. In the last case protease inhibitors cocktail (539131 Set 1, Calbiochem) was used for prevention of monAB digestion. Graphs 1, 2 illustrate received sensograms.



Sensogram 1 shows Fig binding with chip's surface C(Feg) = 100 ng/ml



Sonogram 2 shows fibrin binding with chip's surface C(fibrin) = 250 ng/ml

In model experiment with human Fig and fibrin as antigen was shown that: a) the sensitivity of the method was 100 ng/ml and 250 ng/ml, correspondingly and b) the chip can be regenerated with 50% solution of ethylene glycol in HEPES-buffer. The immobilized monAB retained the ability to bind Fig and fibrin after 10 regenerations and storage at +4°C for 30 days.

Thus, using of SAMs with hydrophilic surface, built of thiols with different length of carbohydrogenic chains, effective and simple method of monAB immobilization, mild method of regeneration and protease inhibitors cocktail for monAB protection are basis for preparation of sensitive and stable immunosensore chip for determination of impotent markers of hemostasis.

References

1. Label-free interaction analysis in realtime using surface plasmon resonance – Technology Note, **23**, Biacore systems, 2007, BR-9004-63, 28-9214-39 AA.

2. A New Approach to Generate Thiol-terminated SAMs on Gold – Jing-Jiang Yu. Agilent Technologies, Inc.2007. 5989-7699EN.