Annual Symposium of the Doctoral School of Molecular Medicine, University of Debrecen

September 3, 2013

Cover page: Birth of an idea (2007)
Sculpture by Julian Voss-Andreae based on potassium channel KcsA.
(Photo by Dan Kvitka. Sculpture commissioned and owned by Roderick MacKinnon.)

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Annual Symposium of the Doctoral School of Molecular Medicine (2012/13 academic year)

University of Debrecen
September 3, 2013

Program of the Symposium

Location: F.003-004 Lecture Hall, Life Science Building, University of Debrecen

09:30	**Arrival** (coffe, cake)
09:55	**Welcome address** **László Csernoch**
10:00-12:00	**Section I.** *Chair* **László Csernoch** Head of the Doctoral School Head of the "Physiology and Neurobiology" doctoral program
	Speakers
10:00-10:10	**Áron Krizsán** 2nd year PhD student *supervisor: László Balkay* Image Quality Capabilities of the MiniPET-III small animal PET scanner for molecular imaging
10:15-10:25	**Levente István Lánczi** 3rd year PhD student *supervisor: Ervin Berényi* Comparison of MRI contrast agents' relaxometry properties on low and high field
10:30-10:40	**Gábor Máté** 2nd year PhD student *supervisor: László Galuska* Radiochemical synthesis and characterisation of novel ^{68}Ga-labelled agents
10:45-10:55	**Zoltán Kónya** 3rd year PhD student *supervisor: Ferenc Erdődi* Effect of glucopyranosyl derivatives on protein phosphatases: structure, activation/ inhibition relationships
11:00-11:10	**Éva Katona** 3rd year PhD student *supervisor: Róza Zákány* Investigation of the role of PP2A in the signaling of different malignant human melanoma cell lines

11:15-11:25	**Roland Ádám Takács** 3rd year PhD student *supervisor: Róza Zákány* Investigating the Ca-signalling toolkit of human chondrogenic progenitor cells
11:30-11:40	**Dóra Bodnár** 3rd year PhD student *supervisor: Péter Szentesi* Effects of selenium on the excitation-contraction (EC) coupling of mouse skeletal muscle
11:45-11:55	**Brigitta Domján** 3rd year PhD student *supervisor: Zoltán Krasznai, György Panyi* Exposure to radiofrequency radiation influences specifically the activity of ion channels
12:00-13:00	**Lunch break**
13:00-15:00	**Section II.** *Chair* **László Virág** Head of the “Cell and molecular biology of signal transduction” doctoral program
	Speakers
13:00-13:10	**Zsuzsanna Nagy** predoctor *supervisor: Gabriella Czifra* Investigation of RasGRP3 expression and function on human breast derived ductal adenocarcinoma samples and cell lines
13:15-13:25	**Dániel Horváth** 1st year PhD student *supervisor: Beáta Lontay* Regulation of SNAP25 by protein phosphorylation
13:30-13:40	**Katalin Petrényi** 1st year PhD student *supervisor: Viktor Dombrádi* Does Hal3 have a protein phosphatase Z regulating function in different fungi?
13:45-13:55	**Éva Kerekes** predoctor *supervisor: Viktor Dombrádi* Searching for the function(s) of the CG9238 gene

	in Drosophila
14:00-14:10	**Márta Kerékgyártó** 2nd year PhD student *supervisor: András Guttman* Light-emitting diode induced fluorescence detection design for a pen-shaped cartridge based single CE system
14:15-14:25	**Csaba Váradi** 2nd year PhD student *supervisor: András Guttman* Haptoglobin Glycosylation: Structure Identification and Biomarker Discovery
14:30-14:40	**Attila Oláh** predoctor *supervisor: Tamás Bíró* Down-stream signaling of the cutaneous cannabidiol actions
14:45-14:55	**Imre Lőrinc Szabó** 1st year PhD student *supervisor: Tamás Bíró* Functional presence of the endocannabinoid system in human hair follicle-derived outer root sheath keratinocytes
15:00-15:15	**Coffee break**
15:15-17:00	**Section III.** *Chair* **János Szöllősi** Head of the "Membrane biophysical questions and research methods" doctoral program
	Speakers
15:15-15:25	**Nikolett Vasas** 1st year PhD student *supervisor: Tamás Bíró* Novel fatty acid amide hydrolase inhibitors exert strong anti-inflammatory effects on human keratinocytes
15:30-15:40	**Andrea Aranyász** 4th year dentistry student *supervisors: Attila Oláh, Tamás Bíró* Fatty acid amide hydrolase inhibitors exert complex anti-acne actions in human sebocytes

15:45-15:55	**Gábor Törő** 2nd year PhD student *supervisor: György Haskó* E.coli upregulates CD39 expression in RAW264.7 macrophages
16:00-16:10	**Csilla Somogyi** predoctor *supervisor: Róza Zákány* Polymodal Vanilloid Receptors on differentiating chondrocytes
16:15-16:25	**Tibor Hajdú** 6th year medical student *supervisor: Róza Zákány* Morphological analysis of hyaluronan-signaling "tool-kit" in normal and cutaneous melanoma-related human samples
16:30-16:40	**Adrienn Tóth** 2nd year PhD student *supervisor: László Csernoch* The effect of follistatin on the Ca^{2+} homeostasis of the C_2C_{12} skeletal muscle cells
16:45-16:55	**Kornél Kistamás** 3rd year PhD student *supervisor: János Magyar* Beat-to-beat variability as a novel predictor of cardiac arrhythmias
17:00	**Conclusion** **János Szöllősi**

Abstracts

Section I

Image Quality Capabilities of the MiniPET-III small animal PET scanner for molecular imaging

Áron Krizsán 2nd year PhD student
Department of Nuclear Medicine
Supervisor: László Balkay

Positron Emission Tomography (PET) is a molecular imaging technique, that became an important clinical investigation in oncology and brain studies. Small Animal PET (saPET) scanners gained high impact in drug development and research in the last decade. The MiniPET-II camera, a Photomultiplier Tube (PMT) and LYSO crystal detector based scanner has been developed in our institute in corporation with ATOMKI (Nuclear Research Insitute of the Hungarian National Academy). Recently a new saPET scanner has been developed where the conventional PMT photodetectors were replaced with Silicon Photomultiplier (SiPM) technology. This new generation of detectors has main importance for the hybrid imaging technique called PET/MRI. Photodetectors that are reliable in strong magnetic fields are necessary for such dual modality systems. The imaging capabilities and the reliability of these scanners is an important issue in the saPET field and the main steps to gain these information is summarized in the NEMA guideline released in 2008. We performed the measurements proposed by the NEMA standard on both of the MiniPET scanners. The measurements included the determination of spatial resolution, sensitivity and spill over ratio. The effective transaxial Full Width at Half Maximum (FWHM) spatial resolution found to be 1.3 mm for the MiniPET-II scanner, and 1.2 mm for the MiniPET-III scanner. The Peak Absolute Sensitivity [%] measured to be 1.37 for MiniPET-II, and 1.36 for MiniPET-III. In addition to the spatial resolution and sensitivity measurements, the spill over ratio (SOR) was also determined and found to be 0.14 in air and 0.23 in water for the MiniPET-II, while for the MiniPET-III scanner these values in air and water were 0.14 and 0.22 respectively. The results presented above indicate that the MiniPET-III scanner with new SiPM detector technology has similar imaging capabilities as the conventional PMT based MiniPET-II scanner, however MiniPET-III would be a concept for MRI compatible small animal PET imaging.

Support: ENIAC grant of ''Central Nervous System Imaging'' (Project no.: 120209) and TÁMOP-4.2.2.C-11/1/KONV-2012-0010)

Comparison of MRI contrast agents' relaxometry properties on low and high field

<u>Levente István Lánczi</u> 3rd year PhD student
Department of Biomedical Laboratory and Imaging Science
Supervisor: Ervin Berényi

Purpose

Magnetic resonance imaging (MRI) is one of the most developing fields of medical imaging. Clinically applied contrast agents make this method more advantageous. Our research aim is to compare MRI contrast media relaxometry properties on Earth-field and on clinical MRI with 1.5T magnetic field.

Methods and materials

Experiments were performed on an Earth-field MRI (EFNMR) device and on a 1.5T clinical MRI scanner. Different concentrations of Gd containing contrast agents solved in water were investigated. T1 and T2 relaxation time measurements were performed five times in a row and five times separately as a control on the Earth-field device for each concentration. T1 and T2 mapping were performed on 1.5T. Evaluations were performed using dedicated software and special mapping program packages. Results were summarized into a database.

Results

Relaxation times of contrast agents changed according to the concentrations. Relaxation times were correlated on 1.5T magnetic field and on the Earth's magnetic field, but shortening of relaxation times were noticed even in lower concentrations on Earth-field. T1 relaxation times were significantly different ($p<0,05$) on low and on high magnetic field of contrast agents studied.

Conclusion

Measurements on low concentrations of contrast-media were more accurate on EFNMR comparing to experiments on 1.5T device. The presented research can be a forerunner of developing new contrast media and biomarkers that can support new clinical applications on both low and high field MRI.

Support: TÁMOP-4.2.1/B-09/1/KONV-2010-0007

Radiochemical synthesis and characterisation of novel ^{68}Ga-labelled agents

<u>Gábor Máté</u> 2nd year PhD student
Department of Nuclear Medicine
Supervisor: László Galuska

In recent decades, gallium-68 has become one of the most important positron-source radionuclides shaping the future of PET-radiopharmacy and thus nuclear medicine. Nevertheless, as a metallic compound, ^{68}Ga makes it necessary to introduce chelator moieties in the syntheses of novel radiopharmaceuticals. Moreover, chemical properties of these chelator groups determine quality and utility of ^{68}Ga-PET-diagnostics. Still, clinically available ^{68}Ga-labelled PET-agents involve the less Ga-specific DOTA chelator for neuroendocrine tumour-imaging (^{68}Ga-DOTA-Toc).
As newest results suggest that ^{68}Ga-affinity and specificity can highly be enhanced through the application of a smaller macrocycle ring and pendant arms with phosphinic modification in these chelators, a study of structure – affinity relationship with a varying number of phosphinic groups was planned and performed in our project. Our measurements show that 1,4,7-triazacyclononane based chelators bearing 2 or 3 phosphinic pendant arms are decent frameworks for future radiosyntheses with ^{68}Ga.
Furthermore, an improved methodology of microfluidic ^{68}Ga-labelling with on-line HPLC analysis has been developed and studied resulting in fast synthesis optimisation, excellent radiochemical yield and reproducibility. In order to test the efficiency of our methodology for formulation and the utility of ^{68}Ga-labelled compounds, ^{68}Ga-NODAGA-$(RGD)_2$, an angiogenesis imaging agent was synthesized and tested using small animal PET and a syngenic skip metastasis animal model.

Support: Apáczai Csere János Scholarship*

*This research was realized in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 “National Excellence Program – Elaborating and operating an inland student and researcher personal support system convergence program”. The project was subsidized by the European Union and co-financed by the European Social Fund.

Effect of glucopyranosyl derivatives on protein phosphatases: structure, activation/ inhibition relationships

Zoltán Kónya **3rd year PhD student**
Department of Medical Chemistry
Supervisor: Ferenc Erdődi

The phosphoserine/threonine specific protein phosphatase-1 (PP1) and -2A (PP2A) are two major types of phosphatase, which are responsible for the dephosphorylation of more than 90 % of cellular phosphoproteins. In an attempt to find PP1 and PP2A specific inhibitors we tested inhibitory potency of several penta-O-galloyl-β-D-glucose (PGG) derivatives (tellimagrandin 1, praecoxin B, mahtabin A, pedunculagin, 1,2-Di-O-Galloyl-4,6-HHDP-β-D-glucose) and a few selenoglycosides. Our results show that tellimagrandin 1 (IC_{50}=0,1-0,22 μM) has more potent inhibitory effect on PP1 than does PGG, while inhibitory potency of other derivatives (praecoxin B, mahtabin A, pedunculagin or 1,2-Di-O-Galloyl-4,6-HHDP-β-D-glicosid varies slightly (0,13-4 μM). Inhibition of PP2Ac occurred at ~100-fold higher IC_{50} values for each derivatives compared to that of determined for PP1c. In contrast, selenoglycosides were without effect or increased the activity of PP1c and PP2Ac in a concentration range of 150-400 μM. One selenoglycoside exerted a weak inhibitory effect on PP1c. In cell survival studies tellimagrandin 1 induced cell death in the range of 5-25 μM concentrations, 1,2-Di-O-Galloyl-4,6-HHDP-β-D-glycoside and pedunculagin were effective in higher concentrations (10-100 μM), while up to 100 μM praecoxin B and mahtabin A were without effect. We conclude that the cell death inducing effect of the PGG derivatives are due to a broader biological influence not just phosphatase inhibition. In seleneoglucosides acetylation of glucose hydroxyls and coupling with hydrophobic aromatic ring appear to be requirements for phosphatase inhibition or activation.

Support: OTKA CNK 80709, TÁMOP-4.2.2/B-10/1-2010-0024, TÁMOP-4.2.2/A-11/1/KONV-2012-0025, Apáczai Csere János Scholarship*

*This research was realized in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 “National Excellence Program – Elaborating and operating an inland student and researcher personal support system convergence program”. The project was subsidized by the European Union and co-financed by the European Social Fund.

Investigation of the role of PP2A in the signaling of different malignant human melanoma cell lines

<u>Éva Katona</u> 3rd year PhD student
Department of Anatomy, Histology and Embryology
Supervisor: Róza Zákány

Malignant melanoma arises from the uncontrolled proliferation of melanocytes located in the basal layer of the epidermis. Different familiar and/or environmental factors can induce the transformation of pigment cells into melanoma cells and numerous signalling pathways can become altered during the melanoma formation. It has already been observed that the mutation of the Ser/Thr specific PP2A may have role in the development of various malignancies. For this reason, the aim of our experiments was to study the role of PP2A in different malignant human melanoma cell lines with application of the potent pharmacological inhibitor okadaic acid (OA) in 20nM concentration. WM35 cells represent a non-metastasizing cell line from an RGP phase primary tumour and a highly metastasizing cell line (HT168) was also used. We proved the presence of PP2A in both cell lines and its activity was inhibitable with 20nM OA. Application of OA decreased the migration of both cell lines and altered hyaluronan (HA) production of melanoma cells. Expression of hyaluronan synthase enzymes was reduced in WM35 while increased in HT168 cells. Expression of HA receptors RHAMM and CD44 were decreased by the PP2A inhibition. We also found altered protein expression level of Ser/Thr specific kinases PKA and ERK1/2 after OA treatment and increased level of Sox9, Sox10, and CREB transcription factors.

These results indicate that PP2A mediated mechanisms play role in the HA homeostasis of the investigated melanoma cell lines and PP2A can be involved in the regulation of migration of melanoma cells.

Support: OTKA-CNK80709, Apáczai Csere János Scholarship*,TÁMOP-4.2.2.A-11/1/KONV-2012-0025

*This research was realized in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 "National Excellence Program – Elaborating and operating an inland student and researcher personal support system convergence program". The project was subsidized by the European Union and co-financed by the European Social Fund.

Investigating the Ca-signalling toolkit of human chondrogenic progenitor cells

Roland Ádám Takács 3rd year PhD student
Department of Anatomy, Histology and Embryology
Supervisor: Róza Zákány

Osteoarhritis (OA) is characterised by the gradual degradation and loss of the shock-absorbing articular cartilage lining articulating surfaces in the joint. During early stages of OA, recovery mechanisms are initiated in the affected cartilage by a unique population of articular cartilage resident cells with limited regeneration capacity. These reparative cells have many stem cells-like features. Having a better understanding about the physiology of these cells could help halt disease progression or reverse the course of cartilage degeneration. Calcium has been pointed out to have important differentiation-related roles in various chondrogenic models. Therefore, we aimed to characterise the calcium homeostasis and the 'Ca-toolkit' of these migratory chondrogenic progenitor cells (CPC).

Our studies were carried out in cells loaded with the calcium-sensitive dye Fura-2 with a set-up able to measure single cell calcium levels. The measurements were performed in normal and calcium-free Tyrode's solution at room temperature. Our findings indicate that CPC cells exhibit spontaneous, repetitive calcium transients at a frequency comparable to that reported in human MSCs. According to our measurements, the IC calcium stores proved to be the source of these spontaneous transients. They required IP3-receptor activation, as they were also detected in a calcium-free external environment, but were abolished after depletion of IC calcium stores by SERCA-blockade, or inhibition of the IP3 pathway.

Our results demonstrate the presence of a complex Ca-signalling toolkit in CPC cells. Elucidating the role of these molecular mechanisms in the modulation of the chondrogenic capacity of these cells requires further investigations.

Support: Mec-09/2011, OTKA CNK 80709, TÁMOP-4.2.2/B-10/1-2010-0024

Effects of selenium on the excitation-contraction (EC) coupling of mouse skeletal muscle

Dóra Bodnár 3rd year PhD student
Department of Physiology
Supervisor: Péter Szentesi

It was shown previously that selenium compounds play a significant role in many physiological functions of the organs, although there is a narrow border between the effective and toxic dose. It has an important anti-oxidant effect, plays role in certain cardiovascular disease prevention, and has positive impact on the cognitive properties. However the effect of Se in skeletal muscle have not been studied yet. In our study we examined the effects of different selenium compounds on muscle properties of mice fed with selenate and NanoSel compounds in different concentrations for two weeks. NanoSel produced at the University of Debrecen, Centre of Agricultural Sciences, NanoFood Labor of Bio- and Environmental-genetics Department, which contains 100-500 nm-sized elemental selenium in organic selenium pellets. Selenate bounds to selenoproteins, while NanoSel transformed into selenit *in vivo*, so it's probably less toxic than selenate. The selenium compounds were used in the concentration of 0.5 and 5 ppm. *In vivo* we measured the forepaw strength with Grip-test, and the mice were housed in cages with activity wheels during the feeding process. During our *in vitro* measurements, we measured force in *soleus* and *extensor digitorum longus* (EDL) muscles of the mouse and we detected changes in intracellular Ca^{2+} concentration on single fibers from *flexor digitorum brevis* (FDB) muscle loaded with fluorescence dye. Both forms of selenium in both muscle types significantly increased the amplitude of single twitches in a concentration dependent manner (e.g. in EDL muscle from 99.5±4.7 mN to 127.8±5.1 in case of 0.5ppm selenate). The fatigue of both muscles was reduced by both selenium compounds in the highest concentration during tetanus series (EDL 31±11, 49±5 and 46±1%; soleus 39±11, 63±5 and 51±6% control, selenate and NanoSel, respectively). The resting intracellular calcium concentration of the FDB fibers was identical in all group measured. In contrast the amplitude of the Ca^{2+} transients evoked by KCl depolarization increased significantly from the control 216±22 nM to 326±60 nM in the presence of 0.5ppm NanoSel. We also measured elementary Ca^{2+} release events (ECRE). Our results suggest that selenate and NanoSel improve the contractile properties of skeletal muscles.

Support: TÁMOP-4.2.2/B-10/1-2010-0024

Exposure to radiofrequency radiation influences specifically the activity of ion channels

Brigitta Domján **3rd year PhD student**
Department of Biophysics and Cell Biology
Supervisor: Zoltán Krasznai, György Panyi

Radiofrequency (RF) waves commonly used in RFID identification systems may modulate transmembrane signalling and thus result in unwanted biological effects. In this study we investigated whether exposure to microwave range (2.45 GHz) RF radiation affects the operation of ion channels having different gating mechanisms. We hypothesized that the fluctuating electromagnetic field may affect the voltage sensor of the channels and thus, may influence the properties of the voltage-gated channels ($h\mathrm{Na_v}1.5$, $h\mathrm{K_v}1.3$), but not the non-voltage gated ($h\mathrm{K_{Ca}}3.1$) ion channel. Channels were transiently expressed in tsA201 HEK cells. Na^+ and K^+ currents were measured using the patch-clamp technique in whole cell or outside out configuration during controlled RF exposure. Currents were evoked by voltage ramps from -120mV to +50mV for $\mathrm{Na_v}1.5$ and from -120mV to +100mV for $\mathrm{K_v}1.3$ channels in 140 mM KF-based intracellular solution. KCa3.1 was studied using voltage ramps from -120 to +40mV at -80 mV holding potential and 1 µM free Ca^{2+}-containing K-aspartate intracellular solution. We showed significant shifts in the activation voltage for the $\mathrm{Na_v}1.5$ at RF power levels of 1W and above, and for the $\mathrm{K_v}1.3$ channel at RF power levels of 5W and 10W. RF exposure did not produce significant changes in the current-voltage relationship of the non-voltage gated $\mathrm{K_{Ca}}3.1$ channel. In addition, the activation and inactivation kinetics of the $\mathrm{Na_v}1.5$ currents were affected significantly at high power levels. We conclude that electromagnetic radiation affects the operation of voltage-gated ion channels, but to a different extent in a channel-dependent manner. These effects should be considered in the application of RFID in the healthcare system.

Support: National Development Agency (TECH09, A2 subprogram, RFSUGMED project), TÁMOP-4.2.2/B-10/1-2010-0024 and TÁMOP-4.2.2.A-11/1/KONV-2012-0025

Section II

Investigation of RasGRP3 expression and function on human breast derived ductal adenocarcinoma samples and cell lines

Zsuzsanna Nagy predoctor
Department of Physiology
Supervisor: Gabriella Czifra

RasGEFs such as RasGRP3 mediate the activation of the Ras signaling pathway that is over-activated in many human cancers. The RasGRP3 proteins exert oncogenic effects and overexpression of the proteins is observed in numerous malignant cancer types. In light of this potential oncogenic effect, we have investigated the expression and potential function of RasGRP3 in breast cancer.
The RasGRP3 and phosphoRasGRP3 expressions were examined in human ductal adenocarcinoma derived samples and cell lines (primary: BT-474; metastatic: JIMT-1, MCF7, SK-BR-3, MDA-MB-453, T-47D) both in mRNA (Q-PCR) and protein (Western blot; immunohisto- and cytochemistry) levels. To explore the biological function of the protein, RasGRP3 knockdown cultures were established. To assess the role of RasGRP3 in the viability of cells, annexin-V/PI staining was performed. To clarify the function of the protein in cell proliferation and in the development of chemotherapeutic resistance, CyQuant assay was performed. To observe the RasGRP3 function in tumor formation, the SCID mouse model was used. To investigate the role of the protein in Ras signaling Western blot experiments were performed.
According to our results the expressions of RasGRP3 and its phospho-form were elevated in the tumor samples. The RasGRP3 expression in the primary cell line was significantly lower than in the metastatic ones. The down-regulation of RasGRP3 induced apoptosis and necrosis in MCF7 cells, inhibited cell proliferation of both cell lines and sensitized the T-47D cells to killing by the chemotherapeutic drugs Tamoxifen and Herceptin. *In vivo* tumor growth of both cell lines was decreased. Inhibition of RasGRP3 expression reduced AKT and ERK1/2 phosphorylation downstream from IGF-I or EGF stimulation.
Our results suggest that RasGRP3 may have a role in the pathological behavior of breast cancer cells.

Support: TÁMOP-4.2.2/B-10/1-2010-0024, TÁMOP-4.2.2/A-11/Konv-2012-0025

Regulation of SNAP25 by protein phosphorylation

Dániel Horváth **1st year PhD student**
Department of Medical Chemistry
Supervisor: Beáta Lontay

Myosin phosphatase (PP1M) consists of a protein phosphatase 1 catalytic subunit (PP1c) and a regulatory subunit (myosin phosphatase targeting subunit, MYPT). The latter plays a role in the localization, substrate specificity and regulation of phosphatase activity. Recent studies have proved the widespread localization of PP1M in neurons. It has been shown that inhibition of PP1M decreases, while suppression of RhoA-associated kinase (ROK) activity increases the extent of neurotransmitter release from cortical synaptosomes. These data suggest an important role of these enzymes in the regulation of neurotransmission. Our goal was to investigate the effect of ROK and PP1M on the phosphorylation level of synaptosomal-associated protein of 25 kDa (SNAP25), one of the potential neuronal substrate of these enzymes in B50 neuroblastoma cells and in rat cortical synaptosomes. SNAP25 is a member of the SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor) complex, along with synaptobrevin and syntaxin. The primary role of SNARE complex is to mediate vesicle fusion. We proved that MYPT interacts with PP1c and SNAP25 by immunprecipitation and Far Western analysis. Surface plasmon resonance (SPR) based binding studies suggested a relatively strong interaction between MYPT1 and SNAP25 (K_A= 1.3 x 10^6). Using siRNA knockdown of MYPT1 we observed increased phosphoylation level of Ser187 and Thr138 in SNAP25 in B50 cell lines. Mass spectrometry analysis demonstrated that ROK phosphorylated these residues in SNAP-25 which was dephosphorylated by PP1M. Our data suggest that ROK and PP1M may play a crucial role in the regulation of neurotransmitter release by mediating the phosphorylation of Thr138 and Ser187 in SNAP25.

Support: OTKA PD104878, TÁMOP 4.2.2.A-11/1/KONV-2012-0025, BMC Korea Fund, Magyary and Bolyai Fellowship of Hungarian Academy of Science and Szodoray Fellowship of UDMHSC.

Does Hal3 have a protein phosphatase Z regulating function in different fungi?

Katalin Petrényi **1st year PhD student**
Department of Medical Chemistry
Supervisor: Viktor Dombrádi

Protein phosphatase Z (PPZ) is a fungus specific protein phosphatase. Its first specific inhibitor, the Hal3 protein, was identified in *Saccharomyces cerevisiae* (ScHal3). Later on two more Hal3 homologues were found in *S. cerevisiae*: Vhs3, Cab3, but only Vhs3 inhibited ScPPZ1.
There is only one Hal3 homologue in *Schizosaccharomyces pombe*, SpHal3. It has a modular structure: the N-terminal half of the protein (SpHal3-Nter) is similarity to Hal3, and its C-terminal half resembles thymidylate synthase. In order to reveal the function of the unusual SpHal3 first were expressed the *S. pombe* PPZ homologue (SpPzh1), as well as SpHal3, SpHal3-Nter, and ScHal3 in bacteria, purified the recombinant proteins with affinity chromatography and assayed the phosphatase activity of SpPzh1 with p-nitrophenylphosphate (pNPP). We found that the phosphatase can be inhibited by SpHal3-Nter (although less effectively than with ScHal3), but the full length SpHal3 had no effect. Thus, SpHal3 did not retain its inhibitory capacity in the fission yeast.
Candida albicans represents another example, as it harbors one PPZ phosphatase (CaPpz1) and two Hal3 homologues: CaHal3 and CaCab3. To investigate PPZ regulation in this pathogenic fungus we expressed and purified the full length CaPpz1 protein, its C-terminal catalytic domain (CaPpz1-Cter), and the putative inhibitors (CaHal3 and CaCab3). Than we measured the phosphatase activities of CaPpz1 and CaPpz1-Cter with two substrates: pNPP and ^{32}P-labeled myosin light chain. According to our *in vitro* assays CaHal3 effectively inhibits CaPpz1 and its catalytic domain, but CaCab3 has no effect on the phosphatase activity. Our results demonstrate that PPZ inhibition by Hal3 has been conserved in *C. albicans*.

Support: UD Faculty of Medicine Research Fund (Bridging Fund 2012), ERASMUS, Libra grants, BFU2011-30197-C3-01, TÁMOP-4.2.2/B-10/-1-2010-0024 and TÁMOP-4.2.2.A-11/1/KONV-2012-0025.

Searching for the function(s) of the *CG9238* gene in *Drosophila*

Éva Kerekes predoctor
Department of Medical Chemistry
Supervisor: Viktor Dombrádi

R5 is a subunit of mammalian protein phosphatase 1 (PP1) that regulates glycogen metabolism. We found the *Drosophila CG9238* gene as a candidate as ortholog of R5. *CG9238* protein (R5h) has PP1-, and glycogen binding motif. We verified the function of the domains: all isoforms of the PP1 catalytic subunit interacted with R5h in yeast two-hybrid experiment, and the recombinant R5h was able to bind glycogen in sedimentation experiment. In addition the recombinant R5h inhibited the PP1 activity. Our *in vitro* data suggest: R5h is a glycogen binding subunit of *Drosophila* PP1.
Three mRNAs can be transcribed from *CG9238*. With RT-PCR we detected the shortest and the two longer mRNAs in embryos and females, but only the two longer mRNAs were detected in larvae, pupae and males. The expression pattern correlates with the available EST data. To reveal the function of R5h, we generated a deletion mutant. Contrary to expectation, we found no notable differences in the properties of the glycogen metabolism between the wild type and deletion mutant strains. Consequently, our data do not support the role of R5h in glycogen metabolism.
To further investigate the function of R5h we used strains (P-element insertion, deletion, RNAi) with different R5h expression levels, and measured the lifespan of the fruit flies. We found that strains with moderately reduced expression level lived longer, while the deletion mutant with the lowest mRNA level lived shorter than the control. Thus the functions of *CG9238* are more complex than expected. The *CG9619* (paralog of *CG9238*) RNAi strain lived longer as well. It is possible, that the *CG9619* gene may take over the functions of *CG9238*, which may explain the moderate effect of R5h deletion/silencing on the glycogen metabolism and lifetime.

Support: TÁMOP-4.2.2/B-10/1-2010-0024, TÁMOP-4.2.2.A-11/1/KONV-2012-0025

Light-emitting diode induced fluorescence detection design for a pen-shaped cartridge based single CE system

Márta Kerékgyártó 2nd year PhD student
Horváth Laboratory of Bioseparation Sciences
Supervisor: András Guttman

CGE is a well-established separation technique for the analysis of biologically important molecules such as nucleic acids. The inherent high resolving power, rapid analysis times, excellent detection sensitivity, and quantification capabilities makes this method favorable compared to conventional manual polyacrylamide and agarose slab gel electrophoresis techniques. In this work we introduce a novel single-channel capillary gel electrophoresis system with LED-induced fluorescence detection also utilizing a compact pen-shaped capillary cartridge design for automatic analysis of samples from a 96-well plate. To evaluate the suitability of the system, 1000 genomic DNA(gDNA) samples were analyzed in gel filled capillaries and detected by the microball ended excitation and emission optical fiber based LED-induced fluorescence detection system. Excellent migration time reproducibility of RSD <0.75% was obtained over the course of 1000 runs. The system rapidly distinguished between intact and degraded gDNA samples, therefore provided important information if they could be used for downstream quantitative PCR processing where high-quality intact gDNA was key. We envision that this novel system design will rapidly find new applications in both research and clinical diagnostic laboratories as a highly sensitive and easy to use bio-analytical approach.

Support: OTKA K-81839

Haptoglobin Glycosylation: Structure Identification and Biomarker Discovery

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A capillary electrophoresis based method was used in combination with exoglycosidase digestions to compare the N-glycosylation profile of haptoglobin in normal and pathologic conditions. To assess the biomarker potential of glycosylation changes in various lung diseases, haptoglobin was isolated from plasma samples of healthy, pneumonia, chronic obstructive pulmonary disease (COPD) and lung cancer patients by means of two haptoglobin specific monoclonal antibodies. Haptoglobin N-glycans were then enzymatically released, fluorescently labeled and profiled by capillary electrophoresis. Disease associated changes of core and antennary fucosylation were identified by targeted exoglycosidase digestions and their levels were compared in the different patient groups. Terms such as of core- and arm-fucosylation degree, as well as branching-degree were introduced for easier characterization of the changes and statistical analysis was used to examine which structures are responsible for the observed differences. Increased level of α1-6 fucosylated tri-antennary glycans was found in all disease groups compared to the control. Elevated amounts of core- and arm fucosylation on tetra-antennary glycans were detected in the lung cancer group compared to the COPD group. A larger scale study is necessary to confirm and validate these preliminary findings in the glycosylation changes of haptoglobin, so could then be used as biomarkers in the diagnosis of malignant and inflammatory lung diseases.

Support: OTKA K-81839

Down-stream signaling of the cutaneous cannabidiol actions

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Supervisor: Tamás Bíró

We have previously shown that the non-psychotropic phytocannabinoid cannabidiol (CBD) exerted complex "cellular" anti-acne actions on human SZ95 sebocytes (normalized "pro-acne agents"-induced lipogenesis, decreased cellular proliferation, and induced universal anti-inflammatory effects). We have furthermore shown that lipid-suppressing activity and anti-proliferative effect were mediated by the activation of the transient receptor potential vanilloid-4 (TRPV4) ion channels; yet, we did not possess any data on mechanism of the anti-inflammatory action. Therefore, in the current study, we aimed at further dissecting its signaling pathway.
Interestingly, we found that anti-inflammatory action of CBD was a TRPV4-independent process, involving direct inhibition of the p65-NFκB signaling pathway (Western blot), and the up-regulation of tribbles homolog-3 (TRIB3) expression, since RNA_i-mediated silencing of the latter was able to fully prevent it. Finally, our preliminary data suggest that the "most proximal" member of the anti-inflammatory pathway is the adenosine A2a receptor, since in the presence of its specific antagonist ZM241385, CBD was unable to up-regulate TRIB3 or suppress lipopolysaccharide-induced tumor necrosis factor-α expression.
Taken together, our results demonstrate that CBD exert its unique, "triple" anti-acne (lipostatic, anti-proliferative and anti-inflammatory) action via activating independent, parallel signaling pathways. Our results highlight the emerging need of systematic exploration of the putative "non-classical" cannabinoid targets in mediating cannabinoid actions in the human skin.

Support: "Lendület" LP2011-003/2011, Jedlik Ányos Predoctoral Scholarship*, TÁMOP 4.2.1./B-09/1/KONV-2010-0007, TÁMOP-4.2.2./A-11/1/KONV-2012-0025, OTKA 101761, OTKA 105369

*This research was realized in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 "National Excellence Program – Elaborating and operating an inland student and researcher personal support system convergence program". The project was subsidized by the European Union and co-financed by the European Social Fund.

Functional presence of the endocannabinoid system in human hair follicle-derived outer root sheath keratinocytes

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We have previously shown that the endocannabinoid anandamide (AEA) inhibits hair shaft elongation and induces catagen regression of human hair follicles (HFs) via the activation of cannabinoid receptor-1 expressed by epithelial cells of the HF. Since HFs were also shown to produce endocannabinoids, we aimed at investigating the functional presence of the endocannabinoid system (ECS) in cultured human HF-derived outer root sheath keratinocytes (ORSKs).

To investigate the proliferation, viability, and cell death, a series of colorimetric (MTT-assay) and fluorescent assays (CyQUANT, $DilC_1(5)$-SYTOX Green staining) were employed. Furthermore, expression of elements of the ECS was identified by immunocytochemistry and quantitative "real-time" PCR (RT-qPCR), whereas alterations in the gene expression levels following various treatments were assessed by RT-qPCR.

Expressions of enzymes involved in the synthesis and degradation of the endocannabinoids were identified in the ORSKs. Moreover, AEA was shown to inhibit cellular proliferation and induce apoptosis-dominated cell death in a concentration-dependent manner. Of further importance, AEA exerted a remarkable anti-inflammatory effect (significant suppression of the basal and/or poly-(I:C)-induced expression of pro-inflammatory cytokines), and induced differentiation. Finally, according to our preliminary results, elevation of the endogenous AEA "tone" of the ORSKs by selective inhibitors of the cellular uptake (AM404) or degradation (URB597) of AEA appeared to mimic the above effects of the exogenously applied AEA.

Support: "Lendület" LP2011-003/2011, TÁMOP 4.2.1./B-09/1/KONV-2010-0007, TÁMOP-4.2.2./A-11/1/KONV-2012-0025, OTKA 101761, OTKA 105369

Section III

Novel fatty acid amide hydrolase inhibitors exert strong anti-inflammatory effects on human keratinocytes

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It has recently been described that endocannabinoids (eCBs) play an essential role in the regulation of the murine skin immune system, and that the elevation of the cutaneous eCB tone, by the inhibition of the eCB degrading enzyme fatty acid amide hydrolase (FAAH), results in anti-inflammatory effects. Therefore, in the current study, we aimed at exploring the putative anti-inflammatory actions of two newly developed FAAH-inhibitors on primary and immortalized human epidermal keratinocytes (KCs).

By treating the KCs with the Toll-like receptor-2 activator lipoteichoic acid (LTA), we first established a "cellular pro-inflammatory model", and we identified certain target genes (interleukin [IL]-1α, IL1β, IL6, IL8), whose expressional alterations specifically indicate the development of the cellular inflammatory response of the KCs. By using this model, we found that both novel FAAH-inhibitors, as well as the commercially available blocker URB597, exerted remarkable anti-inflammatory effects. Indeed, when applied at non-cytotoxic concentrations, they were able to prevent the "pro-inflammatory" actions of LTA to up-regulate the mRNA expressions of the above markers as well as the LTA-induced release of IL6 and IL8. Since these effects were effectively abrogated by the antagonists of CB1 and CB2 receptors, it can be proposed that they indeed elevated the cutaneous eCB tone. Collectively, our data indicate that the novel FAAH-inhibitors are potent anti-inflammatory agents by targeting the "very first-line" players (i.e. the KCs) of the cutaneous inflammatory responses.

Support: "Lendület" LP2011-003/2011, TÁMOP 4.2.1./B-09/1/KONV-2010-0007, TÁMOP-4.2.2./A-11/1/KONV-2012-0025, OTKA 101761, OTKA 105369

Fatty acid amide hydrolase inhibitors exert complex anti-acne actions in human sebocytes

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We have previously shown that prototypic endocannabinoids (e.g. anandamide [AEA]), also produced by the human sebaceous glands (hSGs), can dramatically elevate sebaceous lipid synthesis. Therefore, in the current study, we aimed at investigating the expression and potential role of the endocannabinoid degrading enzyme fatty acid amide hydrolase (FAAH) in the hSGs.
Using the human immortalized SZ95 sebocyte cell line, we have first shown that FAAH is expressed by the cells (RT-qPCR, Western blot). In contrast to our previous expectations (i.e. elevated sebum synthesis by the increase of the “endocannabinoid tone”) we found that non-cytotoxic doses (determined by MTT-assay) of the FAAH-inhibitors URB597 and JP104 did not alter the basal lipid production of the cells (Oil Red O and fluorescent Nile Red staining). In contrast, at high concentrations (1-10 μM), they were able to significantly suppress the lipogenic effect of AEA. Moreover, both substances inhibited proliferation (CyQUANT assay), decreased expression of molecules (IL-18, TNF-α, IGF-1 receptor) involved in the pathogenesis of acne vulgaris, and suppressed the pro-inflammatory action of lipopolysaccharide.
Collectively, these results strongly argue for that these FAAH inhibitors target multiple steps of the pathogenesis of acne vulgaris and hence should be exploited in future clinical studies as promising, novel anti-acne agents.

Support: “Lendület” LP2011-003/2011, Eötvös Loránd Scholarship*, TÁMOP 4.2.1./B-09/1/KONV-2010-0007, TÁMOP-4.2.2./A-11/1/KONV-2012-0025, OTKA 101761, OTKA 105369

*This research was realized in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 “National Excellence Program – Elaborating and operating an inland student and researcher personal support system convergence program”. The project was subsidized by the European Union and co-financed by the European Social Fund.

E.coli upregulates CD39 expression in RAW264.7 macrophages

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Adenosine is a purine nucleoside signaling molecule that elicits its physiological responses by binding to and activating 4 G protein coupled transmembrane adenosine receptors. Extracellular adenosine accumulates following the release of ATP and ADP, which are metabolized to adenosine by a cascade of ectoenzymes on the cell surface. In the first step the extracellular hydrolysis of ATP and ADP to adenosine monophosphate (AMP) is catalyzed by the ectonucleoside triphosphate diphosphohydrolase 1 (ENTPDase1, CD39). AMP then metabolized to adenosine by 5'- ectonucleotidase (Ecto5'Ntase, CD73). Macrophages are pivotal in detecting bacteria and in initiating the host's immune/inflammatory response. The regulation of CD39 in macrophages exposed to bacteria is poorly understood. To study this regulation we stimulated RAW264.7 murine macrophages with heat inactivated *E. coli* and specific Toll-like and NOD-like receptor agonists. *E. coli* increased CD39 mRNA and cell surface protein expression level. The selective TLR2 agonists Pam3CSK4 and FSL-1, TLR3 agonist poly(I:C), TLR4 agonist LPS, TLR5 agonist flagellin and TLR9 agonist ODN1826, but not the TLR7 agonist ssRNA40 or NOD agonists mimicked the effect of *E. coli* in upregulating CD39 expression. *E. coli* increased luciferase activity when the cells were transfected with a CD39 promoter luciferase construct. Thus, bacteria and their products can upregulate CD39 expression.

Support: OTKA CK 78275

Polymodal Vanilloid Receptors on differentiating chondrocytes

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Supervisor: Róza Zákány

Chondrocytes of articular cartilage are exposed to several stimuli, like alternating osmolarity, shear and compressive stress. The Transient Receptor Potential Vanilloid (TRPV) ion channel 4 is one of the most prominent candidates to transmit these stimuli, since mutations of this channel cause numerous severe skeletal dysplasia. These TRPV channels can be characterised with high sequential and functional similarity, therefore these ion channels are planned to be explored during chondrogenesis.
Our experimental model is a high density culture (HDC), established from the distal limb buds of 4-day-old chicken and 11.5-day-old mouse embryos, where spontaneous chondrogenesis occurs within six-seven days of culturing. In both models the mRNA expression patterns of TRPV receptors are monitored in differentiating and mature chondrocytes. The variety of these Vanilloid Receptors decreases in adult articular chondrocytes. To support the presence of these ion channels at protein level we applied Multiple Reaction Monitoring method.
TRPV channels are thermosensitive; therefore, we studied the temporal expression pattern of these channels at four different temperature values (37°C, 41°C, 43°C, 45°C) with RT-PCR, where TRPV1 displays significant changes. By applying thermal stimuli the intracellular calcium ion concentration was also monitored in these cultures; however these calcium transients did not require the presence of extracellular calcium ions. As TRPV receptors are mechanosensitive ion channels, we monitored their mRNA expressions after mechanical load in both model systems. According to our results, it seems that besides TRPV4, TRPV1 and TRPV3 are also involved in mechanical stimulation.

Support: TÁMOP-4.2.2/B-10/1-2010-0024, OTKA CNK807809 and Mec-9/2011

Morphological analysis of hyaluronan-signaling "tool-kit" in normal and cutaneous melanoma-related human samples

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Supervisor: Róza Zákány

Melanoma of the skin is a malignancy developing from epidermal melanocytes and in case of metastasis formation it has poor curability. Extracellular matrix (ECM) of malignant tumours plays important role in maintenance, vascularisation and expansion of the lesion. Hyaluronan (HA) is a glycosaminoglycan component of ECM which has been proved to promote cell movements either in physiological or pathological conditions. Therefore we aimed to demonstrate HA, synthases of HA (HAS2 and HAS3) and one of its receptor molecules (RHAMM) in normal and tissue samples with melanoma metastases. We also investigated HA-homeostasis of normal human epidermal melanocytes (NHEM). We applied RT-PCR and Western blot on NHEM to verify the presence of the investigated molecules. HAS2, HAS3 and RHAMM were expressed at mRNA level, but only the expression of HAS3 protein was well detectable in Western blots. HA production of NHEM was undetectable with HA-probe. HA-probe revealed only very low level of HA in the skin, while the normal lung, liver and lymph node slides demonstrated certain amount of HA. In the same tissues with melanoma metastases, highly elevated accumulation of HA was detected. Despite the presence of HA, immunohistochemistry of HAS2, HAS3 and RHAMM did not show expression of these proteins in normal tissues. The only exception was HAS3 expression in the skin, which may assume some HA production. In contrast, well visible immunopositive signals were detected in the tissues with metastases. As HAS2 and HAS3 secrete HA with different speed in different molecular length, the difference in HAS expression of melanocytes and melanoma cells may have role in the intense motility. This idea is supported by the strong RHAMM positivity in the metastases.

Support: Mec-9/2011, OTKA CNK80709, TÁMOP-4.2.2.A-11/1/KONV-2012-0025

The effect of follistatin on the Ca^{2+} homeostasis of the C_2C_{12} skeletal muscle cells

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Supervisor: László Csernoch

Myostatin, a member of the transforming growth factor β superfamily has emerged as a potent negative regulator of skeletal muscle growth. During embryogenesis, myostatin is exclusively expressed in skeletal muscle to control the differentiation and proliferation of the myoblasts. It mediates the cell signaling cascade through activin receptors in the muscle. Follistatin (FS) is a high affinity activin-binding protein that can act as an activin antagonist. In our experiments we applied FS and we studied the effect of the elimination of activin A signaling pathway and myostatin on the Ca^{2+} homeostasis in C2C12 skeletal muscle cells. Functional experiments on C2C12 myotubes were performed by measuring the changes in $[Ca^{2+}]_i$ following the stimulation by KCl or caffeine. The amplitude of the caffeine induced-Ca^{2+}-transients were significantly higher in FS-treated cells (378±32 nM) as compared to control ones (241±30; $p<0,004$;). Furthermore, the application of KCl did not modify the amplitude of the Ca^{2+} transients. The effect of FS treatment on the expression pattern of proteins involved in the Ca^{2+}-homeostasis of skeletal muscle was investigated at protein level by Western-blot in different stages of the myoblast differentation. We could detect changes in expression of the sarcoplasmic reticulum calcium pump (SERCA) and ryanodine receptor (RyR) as well. Our results suggest that the effect of FS increased the Ca^{2+} sensitivity of RyR.

Support: TÁMOP-4.2.2.A-11/1/KONV-2012-0025, TÁMOP-4.2.2/B-10/1-2010-0024, OTKA NN-107765, Bólyai fellowship

Beat-to-beat variability as a novel predictor of cardiac arrhythmias

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Supervisor: János Magyar

Canine models suggest that the short term beat-to-beat variability (SBVR) is a better predictor of drug-induced torsades de pointes arrhythmias than the measurement of repolarization prolongation alone. The aim of our work is to study the causes and the modulators of SBVR. Action potentials were recorded with conventional sharp glass microelectrodes on enzymatically dispersed ventricular cells from dog hearts. Special equation is used to assess the beat-to-beat variability of ventricular repolarization duration. Changing the cycle length of stimulation caused significant alterations in the action potential duration (APD90) and in the SBVR. Increasing of the cycle length caused decreased SBVR (from control 1 Hz, APD90 243.72±7.65 ms, SBVR 3.50±0.16 ms to 3.33 Hz, APD90 152.80±5.24 ms, SBVR 2.41±0.18 ms; n=8, $p<0.05$), while decreased cycle length increased the SBVR (0.2 Hz, APD90 308.27±12.69 ms, SBVR 6.32±0.41 ms; n=8, $p<0.05$). Changes in the stimulation protocol with current injection, while the ventricular cells are stimulated by supramaximal square wave pulses at 1 Hz frequency can also alter the parameters of the action potential. -80 pA current injection significantly decreased the APD90 and SBVR (from control 0 pA, APD90 213.47±6.96 ms, SBVR 2.68±0.16 ms to -80 pA, APD90 138.08±11.70 ms, SBVR 1.68±0.17 ms; n=10, $p<0.05$). Injection of +60 pA current caused marked variations in the ventricular repolarization (+60 pA, APD90 386.13±29.64 ms, SBVR 11.52±1.91 ms; n=14, $p<0.05$). Our results suggest that if the action potential is shortened, it is followed by a decreased beat-to-beat variability in this experimental setup. The decreased variability – decreased repolarization instability – is thought to be anti-arrhythmic.

Support: Apáczai Csere János Scholarship* , TÁMOP-4.2.2/B-10/1-2010-0024

*This research was realized in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 "National Excellence Program – Elaborating and operating an inland student and researcher personal support system convergence program". The project was subsidized by the European Union and co-financed by the European Social Fund.

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The symposium was supported by the TÁMOP-4.2.2/B-10/1-2010-0024
project co-financed by the European Union and the European Social Fund.