Annual Symposium of the Doctoral School of Molecular Medicine (Academic Year 2015-2016)

University of Debrecen
September 8, 2016

Program

Date: September 8, 2016

Location: F008-009 Lecture Hall, Life Science Building, University of Debrecen

8:30 Arrival (Coffee, cakes)

8:45 László Csernoch: Welcome address

Session 1
Chair: László Csernoch
Head of the Doctoral School and the "Physiology and Neurobiology" doctoral program

9:00 István Tamás (2nd year PhD student)
Department of Medical Chemistry
THE ROLE OF SMTNL1 IN MUSCLE CELLS METABOLISM
Supervisor: Beáta Lontay

9:15 Ágnes Angyal (3rd year PhD student)
Department of Physiology
OPTIMIZATION OF INFLAMMATORY MODEL SYSTEMS OF HUMAN KERATINOCYTES AND HUMAN CORNEAL EPITHELIAL CELLS
Supervisor: Tamás Bíró

9:30 Csaba Aranyi (3rd year PhD student)
Deparment of Nuclear Medicine
EFFECTIVE CONNECTIVITY ANALYSIS OF BRAIN NETWORKS USING DYNAMIC CAUSAL MODELING
Supervisor: Miklós Emri

9:45 Norbert Balogh (3rd year PhD student)
Department of Physiology
EXAMINATION OF THE ROLE OF THE ENDOCANNABINOID SYSTEM IN HUMAN SKELETAL MUSCLE'S BIOLOGICAL AND PHYSIOLOGICAL PROCESSES
Supervisor: Gabriella Czifra

10:00 Monika Béresová (2nd year PhD student)
Deparment of Nuclear Medicine
TEXTURE ANALYSIS OF BRAIN METASTASIS FROM DIFFERENT PRIMARY TUMORS USING MR IMAGES
Supervisor: László Balkay, Ervin Berényi

10:15 Csilla Bordás (3rd year PhD student)
Department of Physiology
NEURONAL AND ASTROCYTIC COMPONENTS OF NEUROMODULATORY ACTIONS ON THE PEDUNCULOPONTINE NUCLEUS (PPN)
Supervisor: Balázs Pál

Break (15 min) Coffee and drinks

Session 2
Chair: János Szöllősi
Head of the "Membrane biophysical questions and research methods" doctoral program

10:45 Ágota Csóti (2nd year PhD student)
Department of Biophysics and Cell Biology
THE ENGINEERED VM24 ANALOGUE WITH IMPROVED KV1.3 SELECTIVITY
Supervisor: György Panyi

11:00 Tibor Hajdú (3rd year PhD student)
Department of Anatomy, Histology and Embriology
SEPTINS PLAY A ROLE IN MELANOMA CELL MIGRATION THROUGH STORE-OPERATED CA2+ ENTRY (SOCE)
Supervisor: Róza Zákány

11:15 Balázs Kelemen (1st year PhD student)
Department of Physiology
INVESTIGATION OF STEROID SENSITIVE TRANSIENT RECEPTOR POTENTIAL MELASTATIN (TRPM) ION CHANNELS IN HUMAN SKIN DERIVED CELLS
Supervisor: Balázs István Tóth

11:30 Tünde Kovács (2nd year PhD student)
Department of Medical Chemistry
THE EFFECTS OF MICROBIAL METABOLITES ON BREAST CANCER CELLS
Supervisor: Péter Bay

11:45 Zsuzsanna Kovács (3rd year PhD student)
Horváth Csaba Laboratory of Bioseparation Sciences
CAPILLARY ELECTROPHORESIS ANALYSIS OF PARAPROTEIN GLYCOSYATION IN MULTIPLE MYELOMA SAMPLES
Supervisor: András Guttman

12:00-13:00 Buffet Lunch

Session 3
Chair: Viktor Dombrádi

13:00 Arnold Markovics (3rd year PhD student)
Department of Physiology
DIFFERENTIAL EFFECTIVENESS OF SELECTED NON-PSYCHOTROPIC PHYTOCANNABINOIDS ON HUMAN SEBOCYTE FUNCTIONS IMPLICATES THEIR INTRODUCTION IN DRY/SEBORRHEIC SKIN AND ACNE TREATMENT
Supervisor: Tamás Bíró

13:15 Judit Márton (2nd year PhD student)
Department of Medical Chemistry
THE ROLE OF PARP-2 IN CHOLESTEROL HOMEOSTASIS
Supervisor: Péter Bay

13:30 Zoltán Pethő (2nd year PhD student)
Department of Biophysics and Cell Biology
KCA1.1 CHANNEL AUXILLARY BETA SUBUNIT COMPOSITION IN GLIOBLASTOMA MULTIFORME
Supervisor: Zoltán Varga

13:45 Zsolt Regdon (3rd year PhD student)
Department of Medical Chemistry
LPS PRECONDITIONING PROTECTS MACROPHAGES FROM H2O2 INDUCED CELL DEATH BY DOWNREGULATION OF PARP-1
Supervisor: László Virág

14:00 Ildikó Szabó (3rd year PhD student)
Department of Medical Chemistry
PYROPTOSIS IN MACROPHAGES
Supervisor: László Virág

14:15 Máté Szarka (2nd year PhD student)
Horváth Csaba Laboratory of Bioseparation Sciences
AN IMAGE ANALYSIS BASED CAPILLARY ELECTROPHORESYS SYSTEM FOR THE DETECTION OF N-GLYCANS FROM HUMAN SERUM
Supervisor: András Guttman

Break (15 min) Coffee and drinks

Session 4
Chair: László Mátyus
Dean of the Faculty of Medicine, UD

14:45 Timea Szendi-Szatmári (3rd year PhD student)
Department of Biophysics and Cell Biology
HOW DOES THE DEGREE OF LABELING AND FLUOROPHORE SATURATION INFLUENCE THE PROPERTIES OF LABELED ANTIBODIES AND INTENSITY-BASED FRET CALCULATIONS?
Supervisor: Péter Nagy

15:00 Márton Géza Szigeti (2nd year PhD student)
Horváth Csaba Laboratory of Bioseparation Sciences
GLYCOMICAL ANALYSIS OF CIRCULATING TUMOR CELLS USING LAB-ON A CHIP TECHNIQUE: GLYCAN ANALYSIS DEVELOPMENT FOR CAPILLARY ELECTROPHORESIS
Supervisor: András Guttman

15:15 Fereydoon Taheri (3rd year PhD student)
Division Biophysics of Macromolecules, German Cancer Research Center (DKFZ), Heidelberg
INTRACELLULAR DIFFUSION STUDIED BY LIGHT SHEET FLUORESCENCE MICROSCOPY (LSFM)
Supervisor: Jörg Langowski

15:30 Emese Tóth (2nd year PhD student)
Department of Medical Chemistry
NEWLY IDENTIFIED FUNCTIONS OF LIM KINASE-2, AS A CPI-17-LIKE PROTEIN PHOSPHATASE-1 INHIBITORY PROTEIN
Supervisor: Andrea Kiss

15:45 János Vincze (3rd year PhD student)
Department of Physiology
HIGH-SPEED CONFOCAL IMAGING REVEALS COMPLEX CALCIUM TRANSIENTS IN PLATELETS
Supervisor: László Csernoch

16:00 Florina Zákány (2nd year PhD student)
Department of Biophysics and Cell Biology
STATE DEPENDENT MODIFICATION OF MUTANT KV10.1 CHANNELS BY MTS REAGENTS
Supervisor: György Panyi

16:15 Closing remarks

ABSTRACTS

István Tamás (2nd year PhD student)
Department of Medical Chemistry

THE ROLE OF SMTNL1 IN MUSCLE CELLS METABOLISM

Muscle cells show phenotypic plasticity in response to changes in environmental and functional requirements. Each phenotypic state is characterized by the expression of a unique set of structural, contractile, and receptor proteins and isoforms that correlate with differing patterns of gene expression. One of the potential regulator of this process is the smoothelin-like protein 1 (SMTNL1). It contains a calponin homology domain at its C terminus and shares sequence homology with the smoothelin family of smooth muscle-specific proteins. Preliminary data suggest that SMTNL1 may play a role as a transcriptional cofactor, and may regulate the expression of numerous proteins of the insulin signaling pathway (IRS1, IRS2, Akt), and cytokines such as interleukin-6. The secreted IL-6 concentration of SMTNL1-/- mice were significantly higher compared to the wild type. Although the mechanism is still not clear but IL-6 and other inflammatory factors play a role in the pathogenesis of type 1 and type 2 diabetes. IL-6 affects the homeostasis and metabolism of the skeletal muscle. Our goal was to establish a stable SMTNL1 overexpressing cell line, using c2c12 mouse skeletal muscle cells. Differentiated skeletal muscle cells were treated with pregnancy hormones, glucose and insulin to challenge the metabolic properties upon SMTNL1 overexpression, and were analyzed by ELISA and Western blot analysis. SMTNL1 modulates the expression of the insulin signaling pathway elements, such as insulin receptor substrate, Akt protein kinase, glucose transporter 4 proteins. The serine amino acid residue phosphorylation of IRS and Akt proteins are also elevated. The IRS serine phosphorylation is an alternative mechanism compared to tyrosine phosphorylation, which impairs the insulin signaling pathway, leading to insulin resistance. Studying the molecular function of SMTNL1 in muscle cells may lead to potential therapeutic target for treating preeclampsia, premature birth, high blood pressure, diabetes. Our hypothesis is that in muscle cells SMTNL1 regulates the expression of genes involved in insulin signaling, and other pathways related to the metabolism such as IL-6.

Supervisor: Beáta Lontay

Ágnes Angyal (3rd year PhD student)
Department of Physiology

OPTIMIZATION OF INFLAMMATORY MODEL SYSTEMS OF HUMAN KERATINOCYTES AND HUMAN CORNEAL EPITHELIAL CELLS

The most important role of our skin is to protect us against various environmental challenges. This protection is realized by complex cutaneous barrier functions, which are mainly formed by the most abundant cell type of the epidermis, keratinocytes. In addition to their role in barrier formation keratinocytes play an important role in the regulation of skin immunological processes, and their role has been proven in several distinct types of cutaneous inflammation such as atopic dermatitis, viral infections and sunburn. An analogous role if fulfilled by the corneal epithelial cells of the eye, which face similar challenges. Since these pathological processes are most commonly investigated in animal experiments, we aimed at developing easy-to-handle, pro-inflammatory in vitro model systems, in which efficiency of novel anti-inflammatory compounds may be investigated in a more humanized environment.

In our experiments we identified treatment times and read-out parameters that showed the most marked changes to the applied challenges (the synthetic TLR3 agonist polyinosinic-polycytidylic acid [poly(I:C)] to model viral infection, UV irradiation to model sunburn and photokeratitis and for corneal cells a combination of ragweed pollen extract and NADPH to model allergic keratitis). Interestingly in both cell types the production of proinflammatory cytokines IL-1α and –β, IL-6 and IL-8 was increased at the mRNA level, while the release of IL-6 and -8 could also be proven with ELISA.

Based on our data we have successfully established different pro-inflammatory model systems suitable for multifaceted, medium throughput investigation of novel anti-inflammatory compounds on epidermal keratinocytes and human corneal epithelial cells.

Supervisor: Tamás Bíró

Csaba Aranyi (3rd year PhD student)
Deparment of Nuclear Medicine

EFFECTIVE CONNECTIVITY ANALYSIS OF BRAIN NETWORKS USING DYNAMIC CAUSAL MODELING

In brain effective connectivity research the fMRI based Dynamic Causal Modeling is a widely used method to estimate the strength of causal neural interactions of a known brain network model. However, finding the best fitting model is computationally demanding because the model space grows exponentially by the number of nodes in the network. In the literature the usefulness of model search methods is shown, for example greedy equivalent search algorithms. Our aim is to speed up DCM computations and to provide a framework to develop model search, and model space shrinkage algorithms.
To reduce computational barriers we developed rDCM which is a reimplementation of the Matlab based DCM12 algorithm using R and C++ languages and the GNU Scientific Library (GSL). Validation and further tests were applied on the 'attention to motion' dataset provided by the Statistical Parametric Mapping toolbox. This contains an fMRI session of three stimuli (Photic, Motion, Attention) and the reference model contains three regions of interest (V1, V5, SPC). Using fixed DCM model input we created a full model space of 531441 possible combinations of intrinsic connections and modulatory effects. After evaluating the entire multidimensional model space we used Bayes factors to find the best fitting model and compare it to the results of some greedy search strategies.
As our first result we implemented rDCM and validated its computational accuracy to the DCM12 algorithm on a model space of 40 different models. We could reduce DCM estimation times to the 1/4 to 1/5 of the original algorithm. We also utilised computer clusters to take advantage of high performance computing possibilities. Second, we estimated the full model space using rDCM, and implemented some greedy search methods. Our experiments showed that the simple greedy equivalent search methods can approximate the model structure of the best fitting model, while finding a local minima in the model space. With the created framework we will be able to characterize model search methods in our future work.

Supervisor: Miklós Emri

Norbert Balogh (3rd year PhD student)
Department of Physiology

EXAMINATION OF THE ROLE OF THE ENDOCANNABINOID SYSTEM IN HUMAN SKELETAL MUSCLE'S BIOLOGICAL AND PHYSIOLOGICAL PROCESSES

The endocannabinoid system (ECS) is a complex signaling pathway composed by various cannabinoid receptors, endogenous ligands and enzymes which are synthesize and degrade the endocannabinoids. The ECS has been shown to play an important role in the regulation of several physiological processes, including e.g. proliferation and differentiation of the skeletal muscle cells. Furthermore, several endocannabinoids have already been demonstrated to exert remarkable anti-inflammatory effects and to alleviate (auto)immune processes, therefore the dysregulation of the ECS might play role in the pathogenesis of inflammatory muscle diseases (e.g. polymyositis).
Therefore, in the current study we aimed at investigating the expression of the members of the ECS in primary human skeletal muscle cells. Moreover, we also wanted to assess the putative expressional alterations of these selected members in polymyositis (PM; a systemic autoimmune disease, characterized by inflammation-accompanied damage of the skeletal muscles) patients-derived skeletal muscle samples.
By using QPCR, we have already shown that members of the ECS, namely the cannabinoid receptors (CB1, CB2, GPR55, GPR119) and the enzyme system what is responsible for synthesize and degrade the endogenous ligands are indeed expressed in skeletal muscle.
In the next phase of our study, we examined the effects of AEA and 2-AG on the proliferation and differentiation of the cells. Interestingly, we found that both substances significantly decreased the proliferation and differentiation of the myocytes in PPAR receptors dependent manner. We also confirmed our aforementioned results on proliferated and rapidly differentiated C2C12 myoblast cell lines.
Finally, we investigated the expression pattern of the ECS in PM patients-derived skeletal muscle samples. We found that expressions of the aforementioned enzymes and the CB1 receptor were decreased in PM patients as compared to healthy individuals (Q-PCR), while expression of GPR119 showed a reverse Jo-1 antibody (a marker of poor prognosis in PM) positivity dependence (i.e. its expression was reduced in Jo-1+ and increased in Jo-1- patients; Q-PCR and immunohistochemistry).

Supervisor: Gabriella Czifra

Monika Béresová (2nd year PhD student)
Deparment of Nuclear Medicine

TEXTURE ANALYSIS OF BRAIN METASTASIS FROM DIFFERENT PRIMARY TUMORS USING MR IMAGES

Objectives: To determine the origin of brain metastases of lung and breast cancer, computing their heterogeneity parameters by means of texture analysis (TA).
Methods: Fifty-eight patients harboring brain metastases from breast (26) and lung cancer (32) were examined by MR imaging. Slices of brain lesions were manually segmented with the collection of 2D ROIs on contrast-enhanced T1-weighted (CET1) images, and local binary patterns (LBP) maps were created from each region. Segmented lesions were categorized into four ROI groups according to their sizes: (1) 0-1935 mm 2 , (2) 1936-3845 mm 2 , (3) 3846-7700 and (4) 7701-11540 mm 2 . Histogram-based (minimum, maximum, mean, standard deviation and variance), and co-occurrence matrix (contrast, correlation, energy, entropy and homogeneity) features were extracted from the CET1 images and LBP maps. Statistical significance was set to $p<0.004$, and t and Mann-Whitney tests were used.
Results: Contrast, correlation, energy and homogeneity were statistically different heterogeneity parameters (SDHPs) in all subgroups between lung and breast metastasis on LBP maps. For all ROI sizes, CET1 images showed less SDHPs than LBP maps (18 vs. 28). In addition, for both the CET1 images and LBP maps there were no statistically different histogram-based indices in the smallest ROI size. Moreover, we found significantly different values in at most two size ranges in the case of all LBP-based histogram features, except for the median.
Conclusions: LBP-based heterogeneity parameters showed significant differences between the textures of metastatic brain lesions of lung and breast primary tumors. The size of the lesions influenced the results (less SDHPs were found for the smallest ROI size), agreeing with others' results that the value of any TA gets reduced for very small ROI areas.

This work was partially supported by the „Richter Gedeon Talentum Alapítvány

Supervisor: László Balkay, Ervin Berényi

Csilla Bordás (3rd year PhD student)
Department of Physiology

NEURONAL AND ASTROCYTIC COMPONENTS OF NEUROMODULATORY ACTIONS ON THE PEDUNCULOPONTINE NUCLEUS (PPN)

The pedunculopontine nucleus (PPN) is a cholinergic nucleus of the reticular activating system, regulating sleep and wakefulness. This nucleus does not only provide cholinergic fibers for several subcortical areas, but also participates as a target of neuromodulatory actions being responsible for homeostatic sleep regulation. Endogenous and exogenous cannabinoids, as well as cholinergic agonists administered to the pedunculopontine nucleus can both influence sleep.
A possible target of several neuromodulatory actions is the M-current. This slowly activating, non-inactivating ion channel is modulated by several G-protein coupled receptors, e.g. Gq-coupled muscarinic acetylcholine receptors, which inhibit opening of this potassium channel, thus enhance neuronal activity. We demonstrated that the M-current is present on the cholinergic, but absent on non-cholinergic neurons, contributing to the differences in firing properties of these neuronal populations.
Activation of astrocytes by cannabinoid type 1 (CB1) receptor agonists are able to modulate the membrane potential of PPN neurons, even in the presence of blockers of fast synaptic neurotransmission. In the present work we provide evidence that synaptic inputs of PPN neurons are also affected by activation of presynaptic and astrocytic CB1 receptors.
Using slice electrophysiology combined with calcium imaging, optogenetics and immunohistochemistry, we revealed a direct presynaptic inhibitory action on inhibitory postsynaptic currents, along with a mild increase of excitatory postsynaptic currents during CB1 receptor stimulation. Besides inhibition of excitatory and inhibitory neurotransmission through stimulation of presynaptic CB1 receptors, astrocyte- and mGluR-dependent tonic inhibition and excitation also developed.
Our findings demonstrate that several paralel neuronal and astrocyte-dependent neuromodulatory actions are present in the PPN. The neuronal M-current is a selective and powerful regulator of the excitability of cholinergic neurons. Furthermore, endocannabinoids act in the PPN via a dual pathway, consisting of a direct presynaptic and an indirect, astrocyte-mediated component, regulating synaptic strength and neuronal activity.

Supervisor: Balázs Pál

Ágota Csóti (2nd year PhD student)
Department of Biophysics and Cell Biology

THE ENGINEERED VM24 ANALOGUE WITH IMPROVED KV1.3 SELECTIVITY

The ion channels expressed by T lymphocytes play key roles in the control of the membrane potential and calcium signaling.The physiological function of T lymphocytes can be modulated selectively by peptide toxins acting on Kv1.3 K+ channels. Because Kv1.3-specific peptide toxins are considered to have a significant therapeutic potential in the treatment of autoimmune diseases, the discovery of new toxins is highly motivated. Since the voltage-gated K+ channels play key role in the regulation of cellular processes in many tissues especially in excitable cells, non-selective inhibitors have high potential risk of developing side effects upon therapeutic application.

Vm24 is a novel Kv1.3 specific peptide isolated from the venom of scorpion Vaejovis mexicanus smithi. The Vm24 inhibits Kv1.3 with high affinity (Kd=2.9pM). However of the tested channels hKCa3.1. mKv1.1, and hKv1.2 were partially blocked by the peptide at 10nM, whereas the other channels were unaffected. To improve the selectivity of Vm24 on Kv1.3 a synthetic Vm24 analogue have generated by amino acid substitution named sVmKTx.

Using whole-cell patch-clamp technique we tested the affinity of the sVmKTx in different concentration on Kv1.3 ionchannel. Kv1.3 currents were half-blocked by 770pM sVmKTx. We assayed the effects of sVmKTx (100nM) on currents of the following seven K+ channels: hKv1.1, hKv1.2, hKv1.4, hKv1.5, rKv2.1. hKCa3.1, and hKCa1.1. For sVmKTx has no effect on these channels.

We can conclude that expected improvement in selectivity for Kv1.3 was achieved, but was accompanied by decrease in affinity.

Supervisor: György Panyi

Tibor Hajdú (3rd year PhD student)
Department of Anatomy, Histology and Embriology

SEPTINS PLAY A ROLE IN MELANOMA CELL MIGRATION THROUGH STORE-OPERATED CA2+ ENTRY (SOCE)

Septins, considered to be the fourth components of the cytoskeleton, are GTP-binding proteins that form hetero-oligomeric complexes, filaments and higher-order structures in the cell cortex. Septins assemble at plasma membrane domains close to puncta of the endoplasmic reticulum suggesting that they may play role in STIM1-Orai1 interaction. Silencing certain septin types or inhibition of septin assembly can block store-operated Ca2+ entry (SOCE) and downstream signalling. SOCE has been proved playing role in melanoma cells' behaviour. We hypothesized that septins can effect melanoma migration and metastasis formation through SOCE.
Presence of septins 2, 4, 5, Orai1, 2, 3 and STIM1, 2 were proved at mRNA and protein level in the two investigated melanoma cell lines (HT199, WM35). Inhibition of septin assembly with forchlorfenuron (FCF) resulted in morphological changes of the cells, without any detectable reorganization of actin cytoskeleton. We found a striking ten-fold reduction of fibronectin guided migration in Boyden chamber as a result of FCF treatments. PTI measurements recorded SOCE in melanoma cells under control circumstances, but FCF significantly reduced the amplitude of these Ca2+-events. Evaluation of proliferation, apoptosis-necrosis and invasion assays is still in process.
Our results suggest that septin assembly is essential for proper SOCE and both septins and Ca2+-events play role in the chemoattraction guided migration of melanoma cells.

Supervisor: Róza Zákány

Balázs Kelemen (1st year PhD student)
Department of Physiology

INVESTIGATION OF STEROID SENSITIVE TRANSIENT RECEPTOR POTENTIAL MELASTATIN (TRPM) ION CHANNELS IN HUMAN SKIN DERIVED CELLS

The skin is our biggest multisensory organ: It is involved in sensing various physical and chemical stimuli and also plays a fundamental role in the etiology of itch and pain. Polymodal Transient Receptor Potential (TRP) ion channels expressed by cutaneous sensory fibers play essential role in skin's sensory functions. However, non-neuronal skin cells also express TRP channels. TRPM3 was recently introduced as a novel thermosensitive nociceptor of primary sensory neurons but until today, its expression was not studied in the cutaneous non-neural cells. Therefore, we aimed at investigating the expression of the TRPM3 in human skin derived cells.
We found that Pregnenolone sulphate (PS) did not activate keratinocytes and sebocytes, but it evoked dose dependent Ca2+ transients on primary human dermal fibroblasts (HDFs) measured by FURA-2 fluorescent Ca2+ indicator. PS evoked Ca2+ transients were abolished by removing extracellular Ca2+ suggesting the presence of a PS activated Ca2+ permeable ion channel in the plasma membrane.
Surprisingly, CIM0216, an exogenous, highly potent TRPM3 agonist failed to evoke Ca2+ entry which result indicated the presence of another PS sensitive Ca2+ permeable ion channel in HDF cells. PS is an allosteric modulator of NMDA receptors (NMDARs) and it can activate TRPM1, a close relative to TRPM3, as well. Therefore, we characterized the PS activated Ca2+ channel with pharmacological tools. Our results showed that the NMDAR antagonist D-AP5 and the TRPM3 antagonist Isosakuranetin had no effect on the PS evoked Ca2+ entry. Although selective and specific blockers and activators of the TRPM1 are still unknown, in the recent years Zn2+ was described inhibiting TRPM1 but not TRPM3. Our results showed that Zn2+ applied in micro molar concentration range could efficiently inhibit Ca2+ transients evoked by PS in HDF cells. As control, we used HEK293T cells overexpressing mTRPM3α2 and we observed a distinct pharmacological profile. Our results let us to draw the conclusion that PS evoked Ca2+ transients may be mediated by TRPM1. In our current experiments, we are focusing on the investigation of the molecular appearance of the TRPM1 in HDF cells.

Supervisor: Balázs István Tóth

Tünde Kovács (2nd year PhD student)
Department of Medical Chemistry

STUDYING NATURAL SMALL MOLECULE MODULATORS OF BREAST CANCER

Warburg metabolism is a characteristic change in the metabolism of cancer cells that was first described by Otto Warburg in the 1920's. Originally, Warburg suggested that tumor cells suppress mitochondrial oxidation even in the presence of oxygen. In fact, Warburg metabolism is way more complex than the original observation, it is now viewed as a complex rearrangement of metabolism to support highly proliferating cells (e.g. tumor cells) by providing key metabolites to support cell division. It should be noted that the actual metabolic changes are tissue/tumor specific, similarly to the molecular mechanism that brings about these changes. Reverting or enhancing Warburg metabolism in susceptible tumors impacts on tumorigenesis, chemosensitivity and the overall pathology of the tumors. Our aim is to identify small molecule modulators of Warburg metabolism and breast cancer pathology.
We have established a phenotyping pipeline for the identification of such (metabotropic) small molecule modulators. The basis of selection is a capability of the chemicals to slow down the proliferation of multiple established breast cancer cell lines (SRB assay, videomicroscopy). Metabolic changes were characterized by oximetry (Seahorse), RT-qPCR and metabolite analysis. On the course of our studies we realized that such metabotropic molecules exert non-metabolic effects too and can impact on other hallmarks of cancer as well. By extending our experimentation we observed changes in oxidative stress and the ENT transition as well. The molecules that performed well in the in vitro tests were further characterized in in vivo tumor grafting models. The treatment of tumor-bearing mice with the small molecule metabolites changed the behavior of the tumor grafts. These tumors were less invasive, showed less characteristic Warburg and ENT features.

Supervisor: Péter Bay

Zsuzsanna Kovács (3rd year PhD student)
Horváth Csaba Laboratory of Bioseparation Sciences

CAPILLARY ELECTROPHORESIS ANALYSIS OF PARAPROTEIN GLYCOSYATION IN MULTIPLE MYELOMA SAMPLES

Multiple myeloma (MM) is an immedicable malignancy of human plasma cells. Its pathogenesis is poorly understood, and mounting evidence indicates that the bone marrow microenvironment of tumor cells has a prominent role in the malignant process. It was shown that multiple myeloma (MM) IgG paraproteins exhibit unique oligosaccharide profiles. In this paper we report on a novel approach for paraprotein N-glycosylation analysis from intact and papain digested molecules. Capillary electrophoresis with laser induced fluorescence detection (CE-LIF) was used for the analysis of the fluorophore labeled (APTS) glycans.

Plasma IgGs and paraproteins were papain digested (Sigma-Aldrich, St Louis, MO) and partitioned with Protein A, Fab kappa (κ) and Fab lambda (λ) columns (PhyNexux, San Jose, CA). Cleavage effectivity and binding of the Fc portion, Fab κ and Fab λ were analyzed with SDS-PAGE using a Mini-PROTEAN System (Bio-Rad, Hercules, CA) by applying 120 V for 2 hours in 12% denaturing gel. Partitioned IgGs fragments were digested with PNGase F to remove their carbohydrate moieties. The liberated glycans, the maltodextrin ladder and maltose internal standard were all fluorophore labeled by 8-aminopyrene-1,3,6-trisulfonic acid (APTS, Sciex, Brea, CA) at 37°C overnight. The labeled samples were purified with CleanSeq magnetic beads (Beckman Coulter, Indianapolis, IN). Capillary electrophoresis profiling of the APTS-labeled N-glycans was performed in a P/ACE MDQ automated CE instrument (Sciex) equipped with a fluorescent detector using an Ar-ion laser (excitation 488 nm, emission 520 nm). The result showed significant differences in the electropherograms of the total Protein A portion, as well as the Fab κ and Fab λ portions of serum glycans of the normal and untreated and treated MM patients. The normal patient samples contain all the regularly expected IgG glycans. In case of untreated MM patient samples the peaks corresponding to structures FA2G2S1, FA2, FA2G2 decreased. Peaks FA2(3)G1 and FA2BG2 increased, while some of the sialylated glycans A2[6]G1S1, A2[3]G1S1, and A2G2S1 disappeared. In case of treated MM patient samples, peak FA2 increased, while peaks FA2[6]G1, FA2(3)G1, FA2B[6]G1, FA2G2 and A2F(3)1G(4)2 representing structures of FA2[6]G1, FA2[3]G1, FA2B[6]G1, FA2G2 and FA2BG2 decreased, while peak FA2B(3)G1 disappeared.

This study represents a comprehensive N-glycosylation pattern characterization of normal as well as untreated and treated MM patient plasma samples. Comparison of the control Protein A, Fab κ and Fab λ IgG glycan fragments with untreated and treated MM patient samples revealed subtle differences. Based on these preliminary results, the next step will be validation of these potential glycan biomarkers in a larger cohort.

Supervisor: András Guttman

Arnold Markovics (3rd year PhD student)
Department of Physiology

DIFFERENTIAL EFFECTIVENESS OF SELECTED NON-PSYCHOTROPIC PHYTOCANNABINOIDS ON HUMAN SEBOCYTE FUNCTIONS IMPLICATES THEIR INTRODUCTION IN DRY/SEBORRHEIC SKIN AND ACNE TREATMENT

Acne is a common skin disease characterized by elevated sebum production and inflammation of the sebaceous glands. We have previously shown that a non-psychotropic phytocannabinoid ((-)-cannabidiol [CBD]) exerted complex anti-acne effects by normalizing "pro-acne agents"-induced excessive sebaceous lipid production, reducing proliferation and alleviating inflammation in human SZ95 sebocytes. Therefore, in the current study we aimed to explore the putative anti-acne effects of further non-psychotropic phytocannabinoids ((-)-cannabichromene [CBC], (-)-cannabidivarin [CBDV], (-)-cannabigerol [CBG], (-)-cannabigerovarin [CBGV] and (-)-Δ9 -tetrahydrocannabivarin [THCV]). Viability and proliferation of human SZ95 sebocytes were investigated by MTT- and CyQUANT-assays; cell death and lipid synthesis were monitored by DilC1 (5)-SYTOX Green labelling and Nile Red staining, respectively. Inflammatory responses were investigated by monitoring expressions of selected cytokines upon lipopolysaccharide treatment (RT-qPCR, ELISA). Up to 10 μM, the phytocannabinoids only negligibly altered viability of the sebocytes, whereas high doses (≥50 μM) induced apoptosis. Interestingly, basal sebaceous lipid synthesis was differentially modulated by the substances: CBC and THCV suppressed it, CBDV had only minor effects, whereas CBG and CBGV increased it. Importantly, CBC, CBDV and THCV significantly reduced arachidonic acid (AA)-induced "acne-like" lipogenesis. Moreover, THCV suppressed proliferation, and all phytocannabinoids exerted remarkable anti-inflammatory actions. Our data suggest that CBG and CBGV may have potential in the treatment of dry-skin syndrome, whereas CBC, CBDV and especially THCV show promise to become highly efficient, novel anti-acne agents. Moreover, based on their remarkable anti-inflammatory actions, phytocannabinoids could be efficient, yet safe novel tools in the management of cutaneous inflammations.

Supervisor: Tamás Bíró

Judit Márton (2nd year PhD student)
Department of Medical Chemistry

THE ROLE OF PARP-2 IN CHOLESTEROL HOMEOSTASIS

In our previous study we have identified the role of PARP2 in cholesterol homeostasis. Deletion of PARP2 increased hepatic cholesterol levels and reduced serum HDL levels. We have found that knockdown of PARP2 enhanced the activity of SREBP1 promoter, therefore PARP2 is a transcriptional repressor of SREBP1 in the liver and through this it regulates the expression of SREBP1 and SREBP1- dependent genes. (Szántó et. al, 2014).

In the current study we have observed a similar effect in skeletal muscle. We have detected downregulation of cholesterol biosynthesis genes in shPARP2 cells compared to scPARP2 cells. Expression of SREBPs and SREBP-dependent genes were validated by qPCR in scPARP2 and shPARP2 C2C12 cells. We have detected higher expression of SREBPs and SREBP-mediated genes in shPARP2 C2C12 cells and similarly to this findings the mRNA levels of these genes increased in skeletal muscles of PARP2-/- mice more than in PARP2+/+ mice. Both in cells and in live mice cholesterol levels were analyzed, and lack of PARP2 increased content of cholesterol in C2C12 cells and in skeletal muscles of mice too. Localization of SREBPs and SREBP-dependent proteins were characterized in C2C12 cells by cell fractionation followed by Western blotting and by confocal microscopy. All results suggested SREBPs transactivation because of PARP2 depletion. After that the luciferase reporter assay proved the transcriptional effect of PARP2 by promoters of SREBPs.

Higher cholesterol content in murine tissue caused by absence of PARP2 suggested increased endogen anabolic steroid synthesis. For this examination we analyzed the mRNA levels of steroidogenesis genes. Star, Hsd17b11, Srd5a1 and Srd5a2 indicated higher expression both in shPARP2 cells and in PARP2-/- mice. We have detected different luciferase signal by Star and Srd5a1 promoters. Depletion of PARP2 induced promoter activity of Star and Srd5a1. In the future our aim is to investigate the levels of steroid hormones in PARP2+/+ and PARP2-/- murine tissue.

Supervisor: Péter Bay

Zoltán Pethő (2nd year PhD student)
Department of Biophysics and Cell Biology

KCA1.1 CHANNEL AUXILLARY BETA SUBUNIT COMPOSITION IN GLIOBLASTOMA MULTIFORME

Glioblastoma Multiforme (GBM) is the most aggressive glial cancer as well as the most common primary malignant brain tumor. Even in the 21st century, regardless of aggressive treatment, the median survival remains less than two years following diagnosis. Therefore, it is crucial to study the biology including the ion channel profile of GBM in order to develop new therapeutic and diagnostic options for the treatment of this disease. GBM tumor cells and tumor model systems express KCa1.1 (BK, Slo1, MaxiK) and predominantly the gBK transcript variant as their major K+ channel. Moreover, it is known that auxillary β and γ subunits modulate the biophysical properties of the KCa1.1 channel, and have restricted tissue expression. In this study, our aim is to characterize the KCa1.1 β subunit composition in both tumor model systems and in freshly isolated tumor cells. We used cultured tumor cell lines (U87, U251) and freshly isolated tumor samples obtained from the Neurosurgery Department of the University of Debrecen. Following isolation, grade IV. GBM samples were homogenized, cultured, then examined by whole-cell patch-clamp technique. In our patch-clamp experiments of GBM cells we measured the activation and inactivation kinetics of the KCa1.1 channel as well as the pharmacological responses to various KCa1.1 modulators (e.g. arachidonic acid, paxilline).
In accordance with current literature, we observed predominantly KCa1.1 currents on GBM cells showing consistent inhibition by paxilline. Moreover, we could deduce the presence of functional β subunits based on RT-PCR, current kinetics and pharmacological response. In conclusion, our findings also support that KCa1.1 has an important role in GBM pathogenesis and the inhibition of this channel specifically targeted via auxiliary subunits may have a potential therapeutic consequence in the future.

Supervisor: Zoltán Varga

Zsolt Regdon (3rd year PhD student)
Department of Medical Chemistry

LPS PRECONDITIONING PROTECTS MACROPHAGES FROM H2O2 INDUCED CELL DEATH BY DOWNREGULATION OF PARP-1

Macrophages (MFs) represent a heterogeneous group of cells of the innate immune system. Their main functions cover but are not limited to first response to pathogens, induction and resolution of inflammation, clearing of pathogens and cell debris and maintaining tissue homeostasis. Upon LPS stimulation MFs display increased migration, phagocytosis and cytokine release.
Poly(ADP-ribose) polymerases (PARPs) are a family of enzymes catalyzing ADP-ribose transfer to target proteins. Posttranslational modification of these proteins result in modulation of chromatin structure, transcription, replication and DNA repair. PARP-1 also acts as a cofactor of NF-□B, the master regulator of inflammation. Oxidative stress-induced PARP-1 hyperactivation may lead to cell dysfunction and necrotic cell death as demonstrated in various cellular systems.
Here we have investigated the possible role of PARP1 in oxidative stress adaptation of MFs in RAW264.7 macrophage-like cell line and primary mouse bone marrow-derived macrophages (BMDM). Preconditioning of cells with LPS attenuated secretion of TNF-alpha following a secondary LPS stimulus. Furthermore, LPS preconditioned MFs proved more resistant to H2O2 induced cell death (as indicated by MTT and LDH assays). Moreover, we observed that LPS preconditioning resulted in downregulation of PARP1 (mRNA and protein) which is likely to be the underlying mechanism for both observations. Experiments are ongoing to establish the causal link between PARP-1 downregulation and altered responses of LPS-preconditioned macrophages. We also plan to identify the molecular mechanism of LPS-induced PARP-1 downregulation.

Supervisor: László Virág

Ildikó Szabó (3rd year PhD student)
Department of Medical Chemistry

PYROPTOSIS IN MACROPHAGES

Pyroptosis is a controlled form of cell death triggered by a variety of pathogenic stimuli or damage signals that induce inflammasome activation. Pyroptotic cells display a rapid loss of membrane integrity that is a result of caspase-1-mediated pore formation in plasma membrane. Plasma membrane pores dissipate cellular ionic gradients producing an increased osmotic pressure, water influx, cell swelling and lysis, followed by release of inflammatory intracellular content. Pyroptosis operates to remove the replication niche of intracellular pathogens, making them susceptible to phagocytosis.
Exposure of macrophages to primary inflammatory stimuli, such as LPS, stimulates pro-IL-1β production. In the presence of secondary stimulus, that triggers caspase-1 activation, macrophages rapidly release large amount of processed IL-1β.
In our pyroptosis model RAW 264.7 macrophage-like cell line and bone marrow derived macrophages are exposed to LPS priming followed by ATP stimulation and cell death parameters are characterized.
Cell death is not affected by incubation of LPS, although cells exhibit an activated state with morphological changes. Exposure to ATP of primed macrophages increases LDH release. Caspase-1 activation is observed in BMDMs which is accompanied with dramatic IL-1β release. Our interest is focused on the role of PARP-1 enzyme in LPS primed and ATP stimulated macrophage cell death.
In RAW macrophages PARP inhibitor PJ-34 and Veliparib significantly decreases LDH release in LPS/ATP treated cells. BMDMs from wild-type and PARP-1 knockout mice relesase different amount of IL-1β and LDH. Our results indicate that PARP-1 or other member of the PARP family has role in our cell death model.

Supervisor: László Virág

Máté Szarka (2nd year PhD student)
Horváth Csaba Laboratory of Bioseparation Sciences

AN IMAGE ANALYSIS BASED CAPILLARY ELECTROPHORESYS SYSTEM FOR THE DETECTION OF N-GLYCANS FROM HUMAN SERUM

One of the most efficient and high-throughput bioseparation methods for therapeutic antibody N-glycan analysis is capillary electrophoresis (CE). The capability to analyze even very low sample amounts made CE an attractive bioanalytical technique. Most of the commercially available capillary electrophoresis systems are using single point detection approaches on the capillary such as photodiode, PMT, laser, etc. This limits the ways of data acquisition to one dimension, thus the results of the data evaluation process. We de veloped a novel, image analysis based, LED induced fluorescence detection system with custom filter algorithms - that is different from the whole-column fluorescence-imaged capillary electrophoresis approach. The system consists of 1) a confocal optical setup, 2) a minicomputer for injection/separation control including a Charge-Coupled Device attached to the optics, and 3) an image analyzing-and-driver software package. The incoming color images are stored and processed on the fly by a channel filter and image calculator operations resulting in mean pixel intensity value plots (electropherograms). Recently developed high sensitivity CCD/CMOS detectors can be readily implemented in CE-LEDIF to increase the resolution by applying different image-analyzing techniques. This includes but not limited to intensity, segmentation or other optical characteristics based property, combined with separation resolution enhancer fluorescent dyes emitting at different wavelengths, e.g., turquoise dye, TEAL, APTS, etc. Storing all collected raw data offers the option for re-processability of the images. It largely contributes to the on-demand refinement of peak intensity by introducing a two-dimensional (array based) detection instead of a one point like measuring technique. Such imaging allows the evaluation of data collected from the injection of highly concentrated or very diluted sample by virtually adjusting the detection window size on the capillary with filtering algorithms, thereby modifying the intensity of the signal without the necessity of reinjecting the sample.

Supervisor: András Guttman

Timea Szendi-Szatmári (3rd year PhD student)
Department of Biophysics and Cell Biology

HOW DOES THE DEGREE OF LABELING AND FLUOROPHORE SATURATION INFLUENCE THE PROPERTIES OF LABELED ANTIBODIES AND INTENSITY-BASED FRET CALCULATIONS?

Fluorescently labeled antibodies are widely used in biological research. Although the degree of labeling or labeling ratio, i.e. the number of fluorophores per antibody, is required in experiments when the fluorescence intensity is to be quantified, its effect on the fluorescence quantum yield and the binding affinity of antibodies has not been characterized in detail. In our experiments, we investigated antibodies with different degrees of labeling. Comparison of the fluorescence intensity of free, unbound and cell-bound antibodies as a function of the labeling ratio implied that the affinity and the quantum yield are influenced by the degree of labeling. We attempted to measure the degree of labeling of non-bound antibodies in solution by photometry, but the measurement was uncertain. Therefore, we examined the anisotropy of antibodies with different degrees of labeling, because homo-FRET occurs between the antibody-bound fluorescent dyes resulting in a decreased fluorescence anisotropy. The anisotropy of the stock solutions of antibodies decreased as a function of the labeling ratio. In order to measure the anisotropy of the cell-bound antibodies we isolated the bound fraction by immunoprecipitation. According to our preliminary results, the anisotropy of the cell-bound antibody fraction is higher than that of the stock solutions suggesting that the degree of labeling of the cell-bound fraction is lower. In our next experiments we would like to examine the anisotropy of additional antibodies, and investigate the effect of the degree of labeling on the fluorescence lifetime.

In another project we examine the effect of fluorophore saturation on the FRET efficiency determined by intensity-based calculations. In confocal microscopy the intensity of exciting light in the focal point of the high numeric aperture objective is so high that the fluorescence of fluorophore saturates meaning that the intensity of fluorescence does not increase proportionally to the increase in the intensity of excitation light. This phenomenon questions the exactness of quantitative fluorescence examinations (e.g. FRET). A manifestation of this phenomenon could be the difference of the FRET values between flow cytometric and microscopic methods since saturation does not takes place in the former. In order to confirm the effect of fluorophore saturation on FRET calculations cells were labeled with fluorescent antibodies against two different epitopes of ErbB2 followed by calculating the hetero-FRET efficiency at different laser intensities in microscopy. The results show that the FRET values decrease with the increase of the laser intensity. We attribute this result to fluorophore saturation. In additional experiments, we will compare the results of flow cytometric and microscopic measurements.

Supervisor: Péter Nagy

Márton Géza Szigeti (2nd year PhD student)
Horváth Csaba Laboratory of Bioseparation Sciences

GLYCOMICAL ANALYSIS OF CIRCULATING TUMOR CELLS USING LAB-ON A CHIP TECHNIQUE: GLYCAN ANALYSIS DEVELOPMENT FOR CAPILLARY ELECTROPHORESIS

There is a growing demand in the biopharmaceutical industry for high throughput and, large scale N-glycosylation profiling of therapeutic antibodies in all phases of product development, but especially during clone selection where hundreds of samples should be analyzed in a short period of time to assure their glycosylation based biological activity. Furthermore, glycoproteins play significant roles in cell development, differentiation and interactions. Thus, analysis of the structural changes of the glycan moieties of these highly complex molecules in living systems may give the opportunity to shed light on the courses of diseases leading to new glycosylation based biomarkers. A magnetic bead based protocol has been developed for N-glycosylation analysis of glycoproteins to alleviate the hard-to-automate centrifugation and vacuum-centrifugation steps of the currently used protocols. Glycan release, fluorophore labeling and clean-up were all optimized resulting in 1 hour magnetic bead based process with excellent yield, and high reproducibility. Capillary electrophoresis analysis of the fluorophore labeled glycans was also optimized for rapid and high resolution separation.
Glycosylation modification research of cancer disease is emphasized for early diagnostic from a simple blood draw. While the entire tumorigenesis progress is not fully understood, it is strongly suggested that cells spreading from the primary tumor play a key role to initiate the metastatic process. Circulating tumor cells (CTC) are defined as cells escaped from the primary tumor and circulating in the cardiovascular system. Design, fabrication, integration and feasibility test results of a novel microfluidic cell capture device is in progress, exploiting the advantages of proton beam writing to make lithographic irradiations under multiple target tilting angles and UV lithography to easily reproduce large area structures. The proposed microstructures were capable to support adequate distribution of body fluids, such as blood, spinal fluid, etc., between the inlet and outlet of the microfluidic sample reservoirs, offering advanced cell capture capability on the functionalized surfaces.

Supervisor: András Guttman

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INTRACELLULAR DIFFUSION STUDIED BY LIGHT SHEET FLUORESCENCE MICROSCOPY (LSFM)

Molecules in living cells are primarily transported by random diffusion. Within individual compartments, such as the cell nucleus, mitochondria or the cytosol, biological macromolecules find their targets mostly by this thermally driven random motion which is fundamental to many intracellular processes such as proteins interaction, enzymatic reaction and signaling as well as pattern and domain formation. In the cellular interior, diffusion is obstructed by crowding and the dense packing of all cellular constituents [Fig1]; this viscoelastic environment has been shown to influence the type of transport observed in the cell significantly [2]. Here we investigated viscoelasticity in the dynamic polymer network of the cell nucleus to gain a better understanding of the underlying physics by recording fast image series of two-dimensional sections of live cells and monitoring diffusion processes in real time. To this purpose we used light sheet fluorescence microscopy, which allows fluorescence (cross-) correlation spectroscopy on all pixels of the entire illuminated plane simultaneously, as well as spatial cross correlation analysis between distant pixels. We studied the mobility of fluorescently labeled histones inside the nucleus of mouse adult fibroblasts in the presence and absence of lamin A, an intermediate filament protein, which provides stability to the nuclear envelope and to the chromatin network. Furthermore, we have applied the same method to artificial model systems of fluorescent nanoparticles embedded in low melting agarose gels of various concentrations, to analyze spatio-temporal dynamics of simple polymer networks. Our data clearly shows the existence of correlated movement both in cells and model systems. Our approach constitutes a framework to systematic and quantitative analysis of diffusion of macromolecules based on the viscoelastic properties of their surrounding environment.

Supervisor: Jörg Langowski

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Department of Medical Chemistry

NEWLY IDENTIFIED FUNCTIONS OF LIM KINASE-2, AS A CPI-17-LIKE PROTEIN PHOSPHATASE-1 INHIBITORY PROTEIN

Retinoblastoma protein (pRb) has a key role in cell survival and proliferation. We have shown, that myosin phosphatase (MP) – including protein phosphatase-1 (PP1) catalytic- and MP target subunit (MYPT1) – takes part in dephosphorylation of pRb. We detected relatively high pRb phosphorylation in untreated THP-1 cells, which may refer to increased level of MP inhibitory proteins. Hence, we tested the presence of a specific MP inhibitory protein – phosphorylated CPI-17 – in THP-1 cells and we found relatively low level of it. But moreover, several other proteins were cross-reacted with the anti-phospho-CPI-17 antibody in higher molecular mass range. In the presence of calyculin-A – applied in PP2A inhibitory concentration – the phosphorylation state of these proteins increased notably. This phosphorylation was attenuated by protein kinase C (PKC), but not by Rho-kinase (ROK) inhibitors, suggesting a PKC-dependent phosphorylation process. Search in protein databases highlighted, that LIMK2 shows significant sequence similarity to the inhibitory phosphorylation site of CPI-17, raising its possible role in PP1 inhibition. To test this hypothesis, Flag-LIMK2 was overexpressed in tsA201 cells, immobilized to Flag-affinity column, phosphorylated in vitro by PKC, then the phosphorylation at the PP1 inhibitory site was confirmed by Western blot, using anti-phospho-CPI-17 antibody. To examine the effect of PKC and ROK on the CLA induced phosphorylation of Flag-LIMK2, tsA201 cells overexpressing Flag-LIMK2 were pretreated with PKC or ROK inhibitors, treated with CLA, then Flag-LIMK2 was immobilized to Flag-beads and its phosphorylation at the inhibitory sequence was verified using anti-phospho-CPI-17 antibody. Phosphatase activity of PP1 was measured in the presence of unphosphorylated and phosphorylated Flag-LIMK2, using myosin light chain substrate. Phosphorylated Flag-LIMK2 decreased the activity of PP1 notably, in a concentration-dependent manner, while unphosphorylated protein had only slight effect.
Our results suggest that – besides its kinase function - LIMK2 may be a novel inhibitor of PP1 and may mediate the phosphorylation level and activity of proteins important in the regulation of tumorigenesis.

Supervisor: Andrea Kiss

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Department of Physiology

HIGH-SPEED CONFOCAL IMAGING REVEALS COMPLEX CALCIUM TRANSIENTS IN PLATELETS

Intracellular calcium transients have long been known to play an important role in platelet activation. The main processes involved are the IP3 mediated release of calcium from the endoplasmic reticulum and the consequent activation of the store-operated calcium entry (SOCE) mechanism, consisting of the STIM-Orai system and TRPC channels. Traditionally the following methods have been used to measure the calcium level in platelets: flow cytometry, spectrofluorimetry and fluorescent microscopy, with Fura-2-AM used as a calcium indicator. These methods, however, are either incapable of visualising the time course of the calcium transient for the same cell during the activation process or show only the composite calcium level for all the cells.
Combining co-loading with Fluo-4-AM and Fura-Red-AM and high-speed confocal imaging (Zeiss LSM Live) we have been able to visualize the calcium transients in case of platelet activation. We investigated the effects of thrombin receptor activating peptide (TRAP), acting on the receptor involved in the physiological platelet activation.
Our results indicate that cellular calcium response to TRAP was highly heterogeneous, with only 22.2% (95% CI: 15.1-30.8%) of the platelets exhibiting a calcium transient after activation by TRAP. The non-responding cells still showed an increase of intracellular calcium level when calcium ionophore A23187 was applied. By first using a calcium-free buffer and re-adding calcium after TRAP activation we were also able to distinctly measure SOCE on these cells. Also, by directly activating IP3 receptors with thimerosal, we have been able to measure the effects of inhibitors of the phospholipase C pathway on SOCE.
We believe that the proposed imaging method is useful for studying the calcium homeostasis of platelets and possibly other blood cells. The observed heterogeneity of platelet responses may have important clinical and pharmacological consequences.

Supervisor: László Csernoch

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Department of Biophysics and Cell Biology

STATE DEPENDENT MODIFICATION OF MUTANT KV10.1 CHANNELS BY MTS REAGENTS

The role of Kv10.1 channels in tumor biology has become evident during the past years. Based on previous studies the gating of Kv10.1 channels remained intact after interrupting the covalent S4-S5 linker between the voltage sensor domain (VSD) and the pore domain (PD). Depending on the site of this interruption mutant split channels exhibit variable electrophysiological properties. While the voltage dependency of conductance of the Y347 split is similar to the wild type channel, L341 split stays at the open state at negative voltages, while Δ342-348 split remains closed at +40 mV. In contrast the VCF measurements have shown no differences between the movement of the VSDs.
To explain the differences in the electrophysiological properties of the splits we hypothesized that the resting position of the VSDs are different among the channels. To probe the VSD positions in the closed state we performed a cysteine accessibility assay to MTSET reagent. Conjugation of MTSET to cysteines substituted at relevant positions in the S3-S4 loop alters channel gating that can be monitored by ion current measurements. We expected that if the resting position of the VSD is different between the full-length and split channels, we would observe changes in accessibility of cysteines substituted in the S3-S4 loop. The RNAs of the channels were injected into Xenopus oocytes, current recordings were performed by TEVC technique. First we assessed the voltage-dependency of the accessibility of full length channel substituted by cysteins at positions 318-326. We have determined the amplitude of the current and tail current, activation and deactivation time constants, G-V shift, Cole-Moore shift before and after 200 μM MTSET application in the closed state. According to the G-V shifts the 319C and the 322C positions can be conjugated by MTSET in the closed state of the full-length channel.
Surprisingly, the modification rates were very similar between the full-length channel and L341 split for both the weakly voltage-dependent I319C site and the strongly voltage-dependent L322C site, suggesting that the two VSDs undergo similar conformational changes, as also reported by VCF.

Supervisor: György Panyi