Annual Symposium of the Doctoral School of Molecular Medicine (Academic Year 2018-2019)

University of Debrecen

September 3, 2019

PROGRAM

Date: September 3, 2019

Location: Lecture Hall, In Vitro Diagnostics Institute (IVDI), University of Debrecen

8:15 Arrival

8:25 László Csernoch: Welcome address

<u>Session 1</u>

Chair: Prof. László Csernoch

Head of the Doctoral School, Head of "Physiology and Neurobiology" Program

8:30 Azzam Aladdin (3rd year PhD student)

TTRACKING OF EXTRA-NEURAL MANIFESTATIONS OF HUNTINGTON'S DISEASE IN PATIENTS DERIVED FIBROBLASTS

Supervisor: Krisztina Tar, Róbert Király

8:45 Dána Al-Gaadi (3rd year PhD student)

CALCIUM HOMEOSTASIS IS MODIFIED IN SKELETAL MUSCLE FIBERS OF SMALL ANKYRIN1 KNOCKOUT MICE

Supervisor: Péter Szentesi

9:00 Beáta Borza (3rd year PhD student)

COMPARISON OF THE N-LINKED CARBOHYDRATES OF BIOLOGICAL THERAPEUTICS AND THEIR BIOSIMILAR VERSIONS

Supervisor: András Guttman

9:15 Karolina Cseri (3rd year PhD student)

MYOCYTE-MACROPHAGE COCULTURE AS AN IN VITRO MODEL FOR INFLAMMATORY MYOPATHIES

Supervisors: László Csernoch, Szilvia Benkő

9:30 Gyula Diszházi (3rd year PhD student)

DANTROLENE REQUIRES MG2+ AND ATP TO INHIBIT THE RYANODINE RECEPTOR.

Supervisor: János Almássy

9:45 Abdennour Douida (3rd year PhD student)

THE PROTEASOME ACTIVATOR PA200 REGULATES CELL SURVIVAL UPON SELECTIVE MITOCHONDRIA INHIBITIONS IN NEUROBLASTOMA CELLS

Supervisor: Krisztina Tar

10:00 Lina Fadel (2nd year PhD student)

IMPACT OF AGONIST TREATMENT ON RXR PARTNER SELECTION

Supervisors: György Vámosi, Gábor Szabó

10:15 Coffe Break

<u>Session 2</u>

Chair: Prof. Péter Bay

"Cell and Molecular Biology of Signal Transduction" Program

10:45 Tímea Hajdu (4th year PhD student)

EXAMINATION OF THE PROTEIN EXPRESSION LEVEL AND THE TYROSINE-KINASE DOMAIN'S FUNCTION UPON THE LIGAND-BINDING OF EPIDERMAL GROWTH FACTOR RECEPTOR

Supervisor: Péter Nagy

11:00 Laura Jankó (3rd year PhD student)

SILENCING OF PARP2 INDUCES AUTOPHAGY

Supervisor: Péter Bay

11:15 Evelin Major (3rd year PhD student)

INVESTIGATION OF THE ROLE OF SMOOTHELIN-LIKE PROTEIN 1 IN HYPERTHYROIDISM

Supervisor: Beáta Lontay

11: 30 Brigitta Mészáros (3rd year PhD student)

COMPARATIVE N-GLYCOME ANALYSIS OF HUMAN SERUM FROM LUNG CANCER, COPD AND THEIR COMORBIDITY PATIENTS BY CAPILLARY ELECTROPHORESIS

Supervisor: András Guttman

11:45 Zsanett Sári (3rd year PhD student)

INDOLEPROPIONIC ACID, A BACTERIAL METABOLITE REDUCES BREAST CANCER AGGRESSIVENES

Supervisor: Péter Bay

12:00 Buffet Lunch

13:00 Poster Session

<u>Session 3</u>

Chair: Prof. János Szöllősi

Head of the "Membrane Biophysical Questions and Research Methods" Program

14:15 Ádám Sipos (3rd year PhD student)

NEW TARGET FOR GLYCOGEN PHOSPHORYLASE INHIBITORS: SODIUM-GLUCOSE CO-TRANSPORTER OF KIDNEY

Supervisor: Tibor Docsa

14:30 Adrienn Skopál (3rd year PhD student)

FUNCTIONAL INTERACTION BETWEEN ADENOSINE 2A RECEPTOR AND CATHEPSIN D PROTEASE IN MACROPHAGES

Supervisor: Endre Kókai

14:45 Krisztina Szabó (3rd year PhD student)

THE ROLE OF PROTEIN PHOSPHATASE Z1 IN OXIDATIVE STRESS RESPONSE OF CANDIDA ALBICANS

Supervisor: Viktor Béla Dombrádi

15:00 Judit Vágó (3rd year PhD student)

CARTILAGE DIFFERENTIATION IN CHICKEN MICROMASS CULTURES ESTABLISHED FROM FORE OR HIND LIMB BUDS SEPARATELY

Supervisor: Róza Zákány

15:15 Closing remarks

ABSTRACTS

Oral Presentations

O1

Azzam Aladdin (3rd year PhD student)

Department of Medical Chemistry

TTRACKING OF EXTRA-NEURAL MANIFESTATIONS OF HUNTINGTON'S DISEASE IN PATIENTS DERIVED FIBROBLASTS

Huntington's disease (HD) is an inherited neurodegenerative disorder, caused by an abnormal polyglutamine (polyQ) expansion in the huntingtin protein (Htt). Mitochondrial dysregulation and impairment of the ubiquitin-proteasome system (UPS) are authentication stamps of HD neurons. The extraneural pathological hallmarks of HD is still not well characterized. We aimed to investigate the crosstalk between mitochondria and proteolytic function in juvenile HD skin fibroblasts which cell type appears an attractive model to study the disease and its pathology. We found reduced mitosis, increased cell size and elevated ROS production in HD fibroblasts compared to healthy control. Cellular viability was similarly maintained. Mitochondrial metabolic analysis did not reveal significant differences compared to control; however, the level of mitochondrial fusion-fission proteins was significantly lower. We hypothesized that HD fibroblasts counterbalance cellular damage and mitochondrial deficit caused by pathological Htt with altered proteasome activity. Our data revealed that HD fibroblasts exhibit higher proteasome activity, which was also associated with elevated gene and protein expression of parkin and a more rapid turnover of the mitochondrial fusion protein Mfn1 relative to control. Our data suggest that HD fibroblasts might respond to mutant polyQ expansion in Htt with altered proteasome activity and faster turnover of specific UPS substrates to maintain cell homeostasis.

Supervisors: Krisztina Tar, Róbert Király

O2

Dána Al-Gaadi (3rd year PhD student)

Department of Physiology

CALCIUM HOMEOSTASIS IS MODIFIED IN SKELETAL MUSCLE FIBERS OF SMALL ANKYRIN1 KNOCKOUT MICE

Small Ankyrins (sAnk1) are muscle-specific isoforms generated by the Ank1 gene that participate in the organization of the sarcoplasmic reticulum (SR) of striated muscles. Accordingly, the volume of SR tubules localized around the myofibrils is strongly reduced in skeletal muscle fibers of 4- and 10-month-old sAnk1 knockout (KO) mice, while additional structural alterations only develop with aging. To verify whether the lack of sAnk1 also alters intracellular Ca2+ handling, cytosolic Ca2+ levels were analyzed in stimulated skeletal muscle fibers from 4- and 10-month-old sAnk1 KO mice. The SR Ca2+ content was reduced in sAnk1 KO mice regardless of age. The amplitude of the Ca2+ transients induced by depolarizing pulses was decreased in myofibers of sAnk1 KO with respect to wild type (WT) fibers, while their voltage dependence was not affected. Furthermore, analysis of spontaneous Ca2+ release events (sparks) on saponin-permeabilized muscle fibers indicated that the frequency of sparks was significantly lower in fibers from 4-month-old KO mice compared to WT. Furthermore, both the amplitude and spatial spread of sparks were significantly smaller in muscle fibers from both 4- and 10-month-old KO mice compared to WT. These data suggest that the absence of sAnk1 results in an impairment of SR Ca2+ release, likely as a consequence of a decreased Ca2+ store due to the reduction of the SR volume in sAnk1 KO muscle fibers.

Supervisor: Péter Szentesi

O3

Beáta Borza (3rd year PhD student)

Research Center for Molecular Medicine

COMPARISON OF THE N-LINKED CARBOHYDRATES OF BIOLOGICAL THERAPEUTICS AND THEIR BIOSIMILAR VERSIONS

Glycoprotein based biotherapeutics are playing a progressively significant role in modern pharmaceuticals. Due to the expiration of patents original biotherapeutics, a new opportunity is open for competing companies to develop their own biosimilar products. Biosimilars are similar to the original products but not exactly the same as the innovator. As a result of the limited information on manufacturing procedures, the reworked process may include some discrepancies such as employed different cell lines and cell culture conditions or purification processes. These changes in the production processes can induce certain minor alterations in the protein structures, e. g., modification of their glycosylation. As the vast majority of recombinant proteins (e.g., monoclonal antibodies) and their biosimilar versions are glycosylated, the attached carbohydrate moiety should be considered as a critical quality attribute and be analyzed during every step of the manufacturing process. Compositional changes in glycosylation of the Fc region can lead to discrepancies in serum half-life, immunogenicity, anti-inflammatory and effector functions. Consequently, it is essential to find adequate analytical methods, which are capable of comprehensively comparing the biosimilar and the original products.

Capillary electrophoresis (CE) with laser induced fluorescent (LIF) detection is an appropriate, accurate and fast method for structural analysis of N-linked carbohydrates, thus can be readily applied for monitoring the slight changes between the original products and their biosimilar counterparts. Also, new, modern multicapillary gel electrophoresis systems are suitable for high-throughput analytical screenings, fulfilling the demands of modern pharmaceutical industry.

Supervisor: András Guttman

O4

Karolina Cseri (3rd year PhD student)

Department of Physiology

MYOCYTE-MACROPHAGE COCULTURE AS AN IN VITRO MODEL FOR INFLAMMATORY MYOPATHIES

Idiopathic inflammatory myopathies are a group of muscle diseases with chronic inflammation. The cause of inflammation is unknown, but an autoimmune origin is presumable in many cases.

High-mobility group box 1 (HMGB1) is a nonhistone protein in the cell nucleus. It is supposed that the mobilized HMBG1 has alarmin function in autoimmune processes and it could be an early inducer of autoimmune myopathy.

In the extracellular milieu HMGB1 can interact with the receptor for advanced glycation end products (RAGE) and toll-like receptors (TLR). These receptors can activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) which evokes expression of inflammatory cytokines, e.g. interleukine 6 (IL6). IL6 was the first cytokine proposed to be a myokine.

In our experiments cocultures of RAW 264.7 macrophage and C2C12 myoblast cell lines were used as an in vitro model for inflammatory myopathy. Localization of HMGB1 was detected with immunofluorescent staining and visualized by fluorescent microscope and a connected camera. IL6 cytokine concentration was determined using enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's protocol.

Our results show that the HMGB1 is localized in the cell nuclei in the proliferation stage of the mono- and cocultures, but with differentiation it progressively disappeared from nuclei of C2C12 of the coculture. In monocultures HMGB1 is still present in the cell nuclei. IL6 production of mono- and cocultures also differs: in monocultures we did not measure considerable level of IL6 production, whilst in the coculture the IL6 concentration of the supernatant is constantly growing from the beginning of differentiation. In case of coculture in the nuclei of C2C12 cells the visibility of HMGB1 gradually weakens parallel with the growing concentration of IL6 in the supernatant.

Our results suggest a strong interaction between temporal and quantitative change of IL6 production and HMGB1 mobilization.

Supervisors: László Csernoch, Szilvia Benkő

O5

Gyula Diszházi (3rd year PhD student)

Department of Physiology

DANTROLENE REQUIRES MG2+ AND ATP TO INHIBIT THE RYANODINE RECEPTOR.

Dantrolene is a ryanodine receptor (RyR) inhibitor, which is used to relax muscles in malignant hyperthermia syndrome. Although dantrolene binds to the RyR protein, its mechanism of action is unknown, mainly because of the controversial data showing that dantrolene inhibited Ca2+-release from intact fibers and sarcoplasmic reticulum (SR) vesicles, but failed to inhibit single ryanodine receptor (RyR) channel currents in bilayers. Accordingly, it was concluded that an important factor for dantrolene`s action was lost during the purification procedure of RyR.

Recently, Mg2+ was demonstrated to be the essential factor for dantrolene to inhibit Ca2+- release in skinned muscle fibers. The aim of the present study was to confirm these results in Ca2+ release- and bilayer experiments, using SR vesicles and solubilized channels, respectively. Our Ca2+-release experiments demonstrated that the effect of dantrolene and Mg2+ was cooperative; and that ATP enhanced the inhibiting effect of dantrolene. Namely, 10 µM dantrolene reduced RyR channel open probability by ~50% in the presence of 3 mM free Mg2+ and 1 mM ATP, while channel activity further decreased to ~20% of control when [ATP] was increased to 2 mM.

Our data provide important complementary information that support the direct, Mg2+-dependent mechanism of dantrolene’s action and suggest that dantrolene also requires ATP to inhibit RyR.

Supervisor: János Almássy

O6

Abdennour Douida (3rd year PhD student)

Department of Medical Chemistry

THE PROTEASOME ACTIVATOR PA200 REGULATES CELL SURVIVAL UPON SELECTIVE MITOCHONDRIA INHIBITIONS IN NEUROBLASTOMA CELLS

Abstract

The conserved Blm10/PA200 activators bind to the proteasome core particle gate and facilitate peptide and protein turnover.Our knowledge about its exact role in diseased cells, however is still limited. Here, we show that stable knockdown of PA200 promotes neuroblastoma cell survival, a significant increase of cell population in S phase, and reduces necrosis after the ATP synthase inhibitor-oligomycin-treatment. However, following rotenone –the complex I inhibitor-exposure, cells exhibit an increase in subG1 and G2/M phases indicative of apoptosis. Using chromatin-immunoprecipitation (ChIP) and ChIP-seq analysis revealed that genes and promoters of which were significantly enriched in PA200, contribute to regulation of crucial intracellular processes such as proliferation, protein modifications and metabolism. In addition, selective mitochondrial inhibitors induced PA200 redistribution in the genome leading to protein withdrawal from some gene promoters and binding to another. Collectively, the results argue for a model in which PA200 fine regulates cellular homeostasis at the transcriptional level, in addition to its described role as an alternative activator of the proteasome. Validation of our obtained bioinformatics data will strengthen this hypothesis and will help to identify the molecular role of PA200 in the transcription machinery

Supervisor: Krisztina Tar

O7

Lina Fadel (2nd year PhD student)

Department of Biophysics and Cell Biology

IMPACT OF AGONIST TREATMENT ON RXR PARTNER SELECTION

Retinoid X Receptor (RXR) plays a pivotal role as a transcription regulator. It serves as an obligatory heterodimerization partner for many other nuclear receptors (NRs). Activation of RXR heterodimers exert a transcriptional activity controlling a wide variety of important biological processes such as development, differentiation, metabolism and cell death. NRs share a common structure composed of several functional domains: an N-terminal transcription activation function domain; a DNA-binding domain; a flexible hinge region domain; and a C-terminal ligand binding domain. The mechanism of activation called molecular switch is also common between these receptors. In this study we intended to understand how the promiscuous RXR molecule behaves in the presence of several potential heterodimeric partners and ligands. We hypothesized that there is a competition between RXR partners for binding to RXR and binding of a specific agonist increases the affinity of a given receptor to RXR. Our hypothesis was tested with three partners of RXR: PPARγ, RAR,VDR using nuclear translocation assay in a three-color model system. The competition was evaluated detecting changes in heterodimerization between RXR and one of its partners, NR1, in the presence of another competing partner, NR2. Therefore, NR1 was needed in a form that is distributed evenly in the cell when expressed alone and enriched in the nucleus when interacting with RXR. These conditions were fulfilled by wt. VDR and mutant forms of both PPARγ and RAR lacking their NLS. Our data revealed that RXR binds to its unliganded partners with different affinities; highest for RAR, lowest for VDR and moderate for PPARγ while specific agonist treatment tips the scale in favor of the liganded partner. This work was undertaken to a better understanding of RXR partition between its different heterodimeric and to obtain insight into triggering specific signaling pathway in complicating cellular environments.

Supervisors: György Vámosi, Gábor Szabó

O8

Tímea Hajdu (4th year PhD student)

Department of Biophysics and Cell Biology

EXAMINATION OF THE PROTEIN EXPRESSION LEVEL AND THE TYROSINE-KINASE DOMAIN'S FUNCTION UPON THE LIGAND-BINDING OF EPIDERMAL GROWTH FACTOR RECEPTOR

The ErbB/HER family of transmembrane receptor tyrosine kinases (RTKs) takes part in various cellular responses activated by EGF and other peptide growth factors. Our aim was to find out if there is some kind of cooperativity between the two active sites of an EGFR dimer and how it depends on the expression level of the receptor and the function of the intracellular kinase domain.

For the experiments A431 (human epidermoid carcinoma), F1-4 (CHO cells stably transfected with ErbB1-GFP) and A4ErbB1 (A431 stably transfected with ErbB1-GFP) cell lines were used. Cells were labelled with fluorescent-EGF and intensities were measured with a FACS Aria flow cytometer.

Expression level of EGFR was decreased via RNA interference. The co-operativity of F1-4 and A4ErbB1 cell populations that showed lower GFP intensity decreased accordingly.

The EGF receptor consists of an extracellular, a transmembrane and an intracellular tyrosine kinase domain. The latter's role in the ligand binding affinity has been studied via treatments with kinase inhibitors. Type-1 (erlotinib, gefitinib) and type-2 (neratinib, lapatinib) inhibitors were added to cells at a concentration of 5 μM. Phosphotyrosine-residues were labelled in EGF stimulated F1-4 cells that were exposed to the inhibitor and fluorescence intensities were examined with an Olympus FV1000 confocal microscope. The effect of these inhibitors was also supported by western blots.

We examined the EGF binding affinity of type-1 and -2 inhibitor exposed F1-4 and A431 cells. According to our results type-1 inhibitor erlotinib that binds to the active conformation of the kinase-domain increased the affinity of EGF-binding. Type-2 inhibitor lapatinib that binds and stabilizes the inactive conformation of the kinase-domain resulted in decreased ligand-binding affinity.

To sum up, the expression level of the receptor influences the co-operativity of EGF-binding and the conformational changes of the intracellular kinase domain of the receptor also plays a role in the affinity of ligand-binding.

Supervisor: Péter Nagy

O9

Laura Jankó (3rd year PhD student)

Department of Medical Chemistry

SILENCING OF PARP2 INDUCES AUTOPHAGY

The poly(ADP-ribose) polymerase enzymes comprise a family of 17 enzymes and poly(ADP-ribose) polymerase 2 (PARP2) belongs to the PARP superfamily. The role of PARP2 was first identified in DNA repair, however, later several other functions of PARP2 were identified in different tissues. PARP2 is a key regulator of cellular metabolism with major impact on mitochondrial metabolism through inhibiting SIRT1 activity.

In our work we used C2C12 and HepG2 scrambled control and PARP2 knockdown cell lines. We examined the expression change of LC3 protein, which is an autophagic marker, moreover, we studied the presence of lysosomes. We assessed the molecular pathways enhancing LC3 expression.

We identified autophagic vesicles in PARP2 knockdown cells using electron microscopy. We used immunofluorescence technique to assess the expression change of LC3 protein. We found that the expression of LC3 increased upon PARP2 depletion, moreover we found an increased lysosomes presence, too. Treatment of shPARP2 C2C12 cells with AICAR (AMPK activator), rapamcyin (mTORC1 inhibitor), olaparib, nicotinamide riboside (both enhance NAD+ levels), and EX-527 (SIRT1 inhibitor), similarly to the silencing of SIRT1 blocked increases in LC3, while resveratrol (SIRT1 activator) induced LC3 expression. We have observed similar changes in shPARP2 HepG2 cells suggesting a general mechanism that is not restricted to C2C12 cells.

Supervisor: Péter Bay

O10

Evelin Major (3rd year PhD student)

Department of Medical Chemistry

INVESTIGATION OF THE ROLE OF SMOOTHELIN-LIKE PROTEIN 1 IN HYPERTHYROIDISM

Skeletal muscle (SKM) is made up of different types of fibers, which are classified into slow oxidative (type1), fast oxidative glycolytic (type2A) and fast glycolytic (type2B). Each fiber type can be remodelled due to hormonal and metabolic changes and by muscle activity. Hyperthyroidism, one of the most common endocrine diseases that is often accompanied by characteristic muscular and metabolic abnormalities, was found to induce fibre type switch from a more oxidative to a more glycolytic phenotype in SKM. We suggest that during type2A/type2B transition, thyroid hormones exert their effect through the smoothelin-like protein 1 (SMTNL1). SMTNL1 has a dual function: it is a negative regulator of myosin phosphatase (MP) in the cytoplasm and upon phosphorylation by protein kinase A (PKA) SMTNL1 translocates to the nucleus and serves as a transcriptional cofactor of steroid receptors. Our results on C2C12 myotubules and human SKM biopsies indicate that in response to elevated thyroid hormone levels the activity of PKA increases, then it phosphorylates SMTNL1 at the Ser301 phosphorylation site which translocates to the nucleus and transcriptionally inhibits the expression of type2A marker Myosin Heavy Chain 2A (MyHC2A), thereby promoting fiber type switch. In addition, Human Phospho-Kinase Array was applied to compare SKM samples of euthyroid and hyperthyroid patients. This array showed significant changes in the phosphorylation levels of several proteins including mTOR, AMPKa2 and STAT5a and b. These changes are in line with our microarray results in which AMPK, JAK/STAT and mTOR signalling pathways were activated or inhibited. Furthermore, we also examined myosin phosphatase (MP) enzyme in our model system. SMTNL1 overexpression and thyroid hormone treatment were found to reduce the expression of MYPT1, the regulatory subunit of MP, while the expression of the catalytic subunit (PP1cδ) is unaffected in C2C12 myotubules. Our findings suggest a possible role of SMTNL1 and MP in mediating skeletal muscle plasticity, through which SKM may be able to adapt to the hyperthyroidism-induced changes.

Supervisor: Beáta Lontay

O11

Brigitta Mészáros (3rd year PhD student)

Research Center for Molecular Medicine

COMPARATIVE N-GLYCOME ANALYSIS OF HUMAN SERUM FROM LUNG CANCER, COPD AND THEIR COMORBIDITY PATIENTS BY CAPILLARY ELECTROPHORESIS

Lung cancer (LC) and chronic obstructive pulmonary disease (COPD) are prevalent pulmonary ailments with high mortality rate in the whole world. It is a great challenge to distinguish LC, COPD, and their comorbidity based on symptoms only, moreover, commonly applied diagnostic methods, including biopsy, are invasive and often serve late results in many cases. It is necessary to develop a non-invasive molecular diagnostic method which could provide early, accurate diagnose (predicting the presence of the actual ailments i.e. lung cancer, COPD or their comorbidity) for optimal treatment. In addition this method also can help to follow the results of the patient treatment process. Recently, glycobiomarker research on serum sample utilization gained increasing importance. In this study pooled human serum samples were investigated by capillary electrophoresis-laser-induced fluorescence assay. Samples were from lung cancer (90), COPD (90) and comorbidity of COPD with lung cancer (90) patients. Sample pooling was applied in order to minimize information loss of species below the detection threshold and improve efficiency of the measurements. In this study 61 N-glycan structures were identified from healthy human serum. The N-glycosylation profiles of the pooled samples were quantitatively compared against pooled sample of healthy individuals. Based on the reported comparative study, a dozen glycan structures were identified as potential glycobiomarker panel, revealing significant changes (>33% relative peak area change) between the pathological and control samples.

Supervisor: András Guttman

O12

Zsanett Sári (3rd year PhD student)

Department of Medical Chemistry

INDOLEPROPIONIC ACID, A BACTERIAL METABOLITE REDUCES BREAST CANCER AGGRESSIVENES

Indolepropionic acid, a bacterial metabolite reduces breast cancer aggressivenes

Zsanett Sári, Edit Mikó, Laura Jankó, Tünde Kovács, Ujlaki Gyula, Péter Bai

In our studies we provided a mechanistic relationship between the microbiome and breast cancer. We assume that bacterial metabolites, released by the microbiome, can reach breast cancer cells through the bloodstream and modulate cancer cells by regulating a set of cancer hallmarks.

Our aim was to assess whether indolepropionic acid (IPA) treatment can influence the behavior of breast cancer cells.

We used cellular and mouse models of breast cancer to examine the effects of indolepropionic acid, a deamination product of tryptophan produced by symbiotic bacteria in the gastrointestinal tract of mammals.

IPA supplementation of Balb/c female mice grafted with 4T1 breast cancer cells resulted in lower tumor mass, lower infiltration of the surrounding tissues, fewer metastases and lower pathological grade of primary tumors. IPA treatment reduced breast cancer cell lines proliferation in the concentrations corresponding to its reference serum concentration range (0.291-1.095 µM). Moreover, IPA reverted epithelial-to-mesenchymal transiton (EMT), decreased the „stem-cell-ness" of rendered cells, and induced oxidative stress in breast cancer cells. We identified that IPA evoked effects are mediated by Aryl Hydrocarbon Receptor (AHR) and Pregnane X Receptor (PXR). AHR and PXR antagonist treatments blocked the effects of IPA on 4T1 breast cancer cells, as well as higher expression of these receptors associated with a prolonged survival of breast cancer patients, based on Kaplan-Meier analyses. In humans we showed whether early stage breast cancer patients, versus control women, had reduced abundance of the Tna gene (bacterial tryptophanse enzyme) in fecal DNA samples, which is responsible for bacterial IPA production. Taken together, these results suggest the relation of the microbiome in the pathogenesis of breast cancer through the decreased biosynthesis of IPA.

Supporters: K108308, GINOP-2.3.2-15-2016-00006, GINOP-2.3.3-15-2016-00021

Supervisor: Péter Bay

O13

Ádám Sipos (3rd year PhD student)

Department of Medical Chemistry

NEW TARGET FOR GLYCOGEN PHOSPHORYLASE INHIBITORS: SODIUM-GLUCOSE CO-TRANSPORTER OF KIDNEY

Diabetes has become widespread and the number of patients is increasing until today. More than 420 million people in the world are afflicted with type 2 diabetes, accounting for ~90-95% of cases. Over time, having too much glucose in your blood can cause health problems such as including cardiovascular disease, neuropathy, retinopathy, and nephropathy. Tight control of blood glucose levels prevents and delays the onset of diabetic complications, but such control is often hard to achieve with oral antidiabetic agents. While many drugs are available for the treatment of type 2 diabetes, glycaemic control remains a problem in diabetic patients and the development of novel drugs is necessary.

Sodium-glucose co-transporter 2 (SGLT2) inhibitors are a new class of diabetic medications indicated only for the treatment of type 2 diabetes. SGLT-s are a group of intracellular glucose transporters in mammalian cells, mediates the glucose reabsorption into the blood. These oral agents treat hyperglycaemia by blocking the reabsorption of glucose in renal tubules, which results in increased urinary glucose excretion and in decreased blood glucose level. However, studies have shown that the risk of diabetes ketoacidosis, urinary tract infection and amputation may be increased in patients taking SGLT2 inhibitors. Thus, there is a need for the development of new SGLT2 inhibitors with less side effects.

The common scaffold of clinically tested SGLT2 inhibitors (among them nine marketed antidiabetic drugs approved in the last six years) consists of a glucose moiety and aromatic rings. Several glucose analogue inhibitors for glycogen phosphorylase (GP) synthesized by our collaborators and tested by our group share a similar structural arrangement. In these compounds, the first aromatic ring is a heteroatomatic ring instead of benzene ring. Therefore, we would like to investigate whether these structurally related compounds could inhibit both GP and SLGT2.The newly synthesized compounds were tested. Compounds with dual inhibition of GP and SGLT2 have been identified and they are tested in liver cells for their ability to reduce the incidence of ketoacidosis.

Supervisor: Tibor Docsa

O14

Adrienn Skopál (3rd year PhD student)

Department of Medical Chemistry

FUNCTIONAL INTERACTION BETWEEN ADENOSINE 2A RECEPTOR AND CATHEPSIN D PROTEASE IN MACROPHAGES

We identified and confirmed the interaction between the C-terminal domain of Adenosine 2A receptor (A2AR) and the Cathepsin D (CtsD) protease in mouse intraperitoneal macrophages (IPMF). The in silico analysis of the primary protein sequence of A2AR predicted several CtsD cleavage sites. These observations and the already published data that CtsD has a role in the degradation of TNFa receptor suggested that CtsD enzyme has similar function in the regulation of A2AR's level. We investigated how the inhibition of aspartyl proteases affects the amount and localization of the A2AR in IPMFs where the interaction earlier has been identified. In order to examine this effect, we immunostained the LPS activated IPMFs with A2AR specific antibody after treatment with the cell permeable aspartyl protease inhibitor (Pepstatin A penetratin). Then the A2A specific fluorescence intensities of the cells were acquired by Leica SP8 confocal microscope and Opera Phenix high throughput confocal microscope. The photos were analyzed with Leica LAS X and Harmony 4.8 software. We have demonstrated that aspartyl protease inhibitor significantly increased the amount, size and intensity of A2AR specific fluorescent spots in murine IPMFs in a concentration-dependent manner both in the membrane and cytoplasmic regions. In order to investigate whether A2AR can be a substrate of CtsD protease, we used a transgenic cell line that overexpresses the A2A receptor. This cell lysate was co-incubated for different time intervals and recombinant CtsD enzyme concentrations. We have shown by western blot that CtsD treatment significantly reduced the amount of A2AR protein in the cell lysate. Furthermore, we demonstrated that inhibition of the aspartic proteases increased not only the intracellular cAMP level but also induced the secretion of anti-inflammatory and suppressed the inflammatory cytokine production. Based on our results, we propose that CtsD protease regulates the amount and signaling of A2AR in IPMFs.

Supervisor: Endre Kókai

O15

Krisztina Szabó (3rd year PhD student)

Department of Medical Chemistry

THE ROLE OF PROTEIN PHOSPHATASE Z1 IN OXIDATIVE STRESS RESPONSE OF CANDIDA ALBICANS

Candida albicans is an opportunistic pathogen which is responsible for nosocomial infections. C. albicans contains a fungus specific serine/threonine protein phosphatase, CaPpz1 that has several important physiological roles such as the regulation of cation homeostasis, cell wall biosynthesis, morphological changes, oxidative stress response, and the virulence of the pathogen. In the present work we investigated the consequences of CaPpz1 phosphatase deletion in the absence and presence of the oxidizing agent, tert-butyl hydoperoxide (tBOOH). First we confirmed that tBOOH reduced the growth rate and blocked the division of the fungal cells without a dramatic modification of their morphology, viability and vitality; both in the cappz1 mutant (KO) and the genetically matching QMY23 (WT) strains. Than we carried out a full transcriptome analysis by RNA sequencing in order to reveal the gene expression changes behind the above mentioned physiological changes. Based on the numbers of genes affected and the amplitudes of the changes observed, we concluded that the deletion of the protein phosphatase enhances the effects of oxidative stress. With the aid of gene ontology enrichment analysis, we selected 64 genes for further validations by RT-qPCR. We confirmed that the expression of genes coding for cytosolic ribosomal proteins and cell surface proteins were downregulated by tBOOH treatment, while the amounts of mRNAs associated with transport processes, oxidoreductase activity, and RNA processing were upregulated. The effects of KO alone were moderate and variable suggesting that the relatively instable response mechanisms elicited by CaPpz1 deletion are stabilized and elevated under oxidative stress conditions. From these results we conclude that in the wild type C. albicans CaPpz1 plays a protective role against oxidative damage, and suggest that the specific inhibition of this phosphatase combined with mild oxidative treatment could be a feasible new approach of antifungal therapy. Our work was supported by the NKFIH K108989 grant, and by the ÚNKP-18-3 New National Excellence Program of the Ministry of Human Capacities.

Supervisor: Viktor Béla Dombrádi

O16

Judit Vágó (3rd year PhD student)

Department of Anatomy, Histology and Embryology

CARTILAGE DIFFERENTIATION IN CHICKEN MICROMASS CULTURES ESTABLISHED FROM FORE OR HIND LIMB BUDS SEPARATELY

Chicken embryos are popular and often-used experimental models in developmental biology researches. Micromass cell culture system derived from mesenchymal cells of the embryo limb buds can serve as a reliable in vitro model for cartilage differentiation and endochondral skeletal development. In order to harvest the required high number of cells, these cell cultures are regularly established from a mixture of fore and hind limb buds. Our objective was to examine the alterations in chondro- and osteogenesis using only the fore or the hind limb buds for cell culture preparation.

Chicken embryos of Hamburger-Hamilton 22-24 stages were used to create primary micromass cell cultures of chondrifying mesenchymal cells. Fore or hind limb buds were collected separately. Cell cultures established from a mixture of fore and hind limb buds served as control groups. Cultures were harvested on the 1st, 3rd, 6th and 9th days of culturing, according to the steps of in vitro chondrogenesis. Changes in the quantity of cartilage-specific extracellular matrix were examined by dimethyl-methylene blue and toluidine blue metachromatic staining methods. qPCR reactions were carried out to study the expression of cartilage-specific (Sox9: SRY-box 9, Col2a1: collagen type II alpha 1 chain, Acan: aggrecan, Has2 and Has3: hyaluronan synthase 2 and 3) and bone-specific marker genes (Runx2: runt related transcription factor 2, Col10a1: collagen type X alpha 1, Alpl: alkaline phosphatase, liver/bone/kidney, Bmp2: bone morphogenetic protein 2, BGLAP: bone gamma-carboxyglutamate protein/osteocalcin).

According to our first results, cell cultures established from only the fore limb buds showed a significantly pronounced and more advanced cartilage formation in comparison with the hind limb and the mixed limb bud derived micromass cultures.

Supervisor: Róza Zákány

ABSTRACTS

Posters

P1

Dóra Antal (1st year PhD student)

Department of Medical Chemistry

PARP-2 ACTIVATION MAY BE A PATHOGENIC FACTOR IN PSORIASIS

Dóra Antal, Annamária Szödényi, Kata Hegyi, Péter Bai, Borbála Kiss, Magdolna Szántó

Psoriasis is an inflammatory skin disease with characteristic changes in the epidermis due to excessive hyperproliferation and abnormal differentiation of keratinocytes, resulting in a thicker epidermis in patients.

PARP-2 is a member of the superfamily of poly(ADP-ribose) polymerase (PARP) proteins. PARP-2 was originally described in connection to DNA repair. We have identified rearrangements in gene expression upon the knockout of PARP-2, which is heavily impact inflammation and metabolism. The function of PARP-2 in the skin is not yet characterized.

A widely used mouse model for psoriasis-like skin inflammation is the topical treatment with imiquimod (IMQ) of the animals. We applied the IMQ-containing Aldara cream on the back skin of PARP-2+/+ and PARP-2-/- mice for 5 days to induce psoriasis-like dermatitis on the animals. The severity of psoriasiform dermatitis were scored daily. Macroscopically, the developed skin disease was significantly less severe in the PARP-2-/- mice than in the PARP-2+/+ mice. Microscopical analyses of the lesions revealed that the thickening of the epidermis was less pronounced in the PARP-2-/- than in the PARP-2+/+ mice. Histochemical analyses of the lesions has shown increased expression of the keratinocyte differentiation-associated protein, keratin 10 in the epidermis of the skin of PARP-2-/- mice both in the IMQ-treated and control groups. This was strengthened in keratinocyte cell cultures, where PARP-2 has been depleted by lentiviral-mediated gene silencing. We found increased apoptotic rate in keratinocytes upon the depletion of PARP-2, which may contribute to the protective effect of PARP-2 knock-down against epidermal thickening in psoriasis-like lesions in mice. Our findings in mice and in vitro cell cultures were further supported by the analyses of skin sections of psoriasis-affected patients, where in situ hybridization detected increased PARP-2 mRNA expression in psoriatic epidermis.

Our results suggest that PARP-2 regulates keratinocyte physiology, and the dysregulation of PARP-2 may contribute to the pathomechanism of psoriasis.

Supervisor(s): Magdolna Szántó

P2

Dorottya Ádám (2nd year PhD student)

Department of Physiology

ACTIVATION OF ?-OPIOID RECEPTOR (KOR) SUPPRESSES PRO-INFLAMMATORY RESPONSE OF HUMAN EPIDERMAL KERATINOCYTES

It has recently been shown that ?-opioid receptor (KOR) is expressed on human epidermal keratinocytes. Moreover, reduction of its expression correlated with the severity of pruritus in psoriatic lesions. These results suggest that homeostatic KOR signaling may act as a gate-keeper in controlling the release of certain pruritogenic and pro-inflammatory mediators from human epidermal keratinocytes. Thus, in the current study, we aimed to assess the role of KOR in regulating inflammatory responses of human epidermal keratinocytes.

Using human immortalized HaCaT keratinocytes, we found that KOR is expressed on these cells on protein level (western blot). Next, we assessed the effects of the selective KOR agonist nalfurafine (NALF), and found that, it did not influence viability (24-48 hrs; MTT-assay) or proliferation (24-72 hrs; CyQUANT-assay) of the keratinocytes up to concentration of 10 µM. In order to exclude the possibility of the onset of early apoptotic or necrotic processes, we also performed DilC1(5)-SYTOX Green double labeling together with glucose-6-phosphate dehydrogenase release assay, which demonstrated that NALF did not initiate cell death of human keratinocytes.

Since NALF can be administered without the risk of cytotoxicity in a wide concentration range, we next probed its efficiency in suppressing the Toll-like receptor 3 activator poly-(I:C)-induced pro-inflammatory response. Of great importance, we found that NALF (10 nM) could abrogate the poly-(I:C)-induced up-regulation of several pro-inflammatory cytokines (namely, interleukin [IL]-1a, IL-1ß, IL-8 and tumor necrosis factor-a; 24-hr treatments; Q-PCR) in a KOR-dependent manner, since this effect could be prevented by co-administration of the KOR-selective inverse agonist nor-BNI (100 nM).

Taken together, our data suggest that KOR may be a potent anti-inflammatory regulator of the epidermal keratinocytes.

Supervisor(s): Attila Oláh

P3

Tsogbadrakh Bayasgalan (2nd year PhD student)

Department of Physiology

THE M-CURRENT IS A POTENTIAL SYNCHRONIZER OF MESENCEPHALIC CHOLINERGIC NEURONS

Bayasgalan T.*, Kovács A.*, Szentesi P, Baksa B, Csemer A., Szücs P., Pál B.

*equal contribution

The pedunculopontine nucleus (PPN) is a cholinergic part of the reticular activating system, which provides cholinergic and non-cholinergic fibers to several subcortical areas. Cholinergic PPN neurons also receive cholinergic inputs, which inhibits the M-current, a voltage-gated potassium current. In the present work, we investigated the presence, subunit composition and functional roles of the M-current in cellular, network and behavioral levels.

Cellular electrophysiological experiments were performed on midbrain slices and thalamus-midbrain blocks, as well as activity wheel test was done on KCNQ4 knockout mice and wild type littermates.

M-current was only present on the cholinergic neurons. Inhibition of the M-current decreased spike frequency adaptation, whereas M-current activators increased it. High threshold membrane potential oscillations were almost completely inhibited by blockade of M-current. M-current activators increased its activation threshold and slightly reduced its amplitude. Optogenetic activation of LDT cholinergic neurons led to M-current inhibition of the PPN cholinergic neurons. Paired recordings of uncoupled neighboring PPN cholinergic neurons revealed that the M-current inhibition decreases the level of spontaneous synchronization between them.

The activity cycles of KCNQ4 knockout mice changed in a significantly different way responding to changes of light-darkness cycles compared to wild type littermates.

One can conclude that the M-current is a hallmark of the cholinergic neurons in the PPN. The channel responsible for M-current is partially, but not exclusively, formed by KCNQ4 subunits. The M-current of PPN cholinergic neurons seem to participate in neuronal synchronization and thus in regulation of PPN activity and sleep-wakefulness cycles.

Supervisor(s): Balázs Pál

P4

Andrea Csemer (1st year PhD student)

Department of Physiology

OREXINERGIC NEUROMODULATORY ACTIONS MODIFY OCCURRENCE OF SLOW INWARD CURRENTS ON NEURONS IN THE PEDUNCULOPONTINE NUCLEUS

Orexins are neuromodulatory peptides of the lateral hypothalamus, which regulate important homeostatic mechanisms including sleep-wakefulness cycles. Orexinergic actions stabilize wakefulness by acting on nuclei of the reticular activating system (RAS) including the pedunculopontine nucleus (PPN). Orexinergic actions in the PPN on cellular level comprise of the development of a tonic inward current or depolarization; mediated by calcium- and mixed cationic conductances, as well as occurrence of noisy background currents and an increase of excitatory postsynaptic current frequency and amplitude.

It was previously shown that serotonergic, cholinergic and cannabinoid actions on the PPN can elicit various responses including depolarization and hyperpolarization. Independently from it, astrocyte-dependent and NMDA receptor mediated ’slow inward currents’ (SICs) were regulated in a way related to the previous SIC activity.

In the present project, we investigated orexinergic neuromodulatory actions on SICs of PPN neurons and their relationships with tonic currents by using slice electrophysiology on preparations from mice. We demonstrated that -in contrast with several other neuromodulatory actions and in line with literature data- orexin almost always elicited a tonic inward current. Independently from the tonic currents, actions on SICs were also detected which resembled to other neuromodulatory actions: if SICs were almost absent on the neuron, orexin induced an increase of the charge movements by SICs, whereas if SIC activity was abundant on the neurons, orexin exerted inhibitory action on it.

This finding might strengthen the theory that an astrocyte-dependent neuromodulatory action exists in the PPN, which uniformly responds to several different actions and sets a certain low level of ’random’ neural activity.

Supervisor(s): Balázs Pál

P5

Csaba Dienes (1st year PhD student)

Department of Physiology

ROLE OF NMDA RECEPTOR IN CANINE LEFT VENTRICULAR CARDIOMYOCYTES

Backround: NMDA receptor (N-Methyl-D-Aspartic acid receptor) is a physiological receptor expressed throughout the body especially in the nervous system but also present in the human heart. Type 1 NMDA receptor (T1NR) is expressed in all areas of heart including the right and the left ventricles. When it gets activated by its agonist, either NMDA itself or glutamate, it will result in cellular excitation due to influx of cations.

Ingestion of MSG, the general flavor enhancer frequently used in Asian food, has been suggested to cause the so called Chinese restaurant syndrome (postprandial tachycardia, arrhythmias, and palpitations) in some individuals. Giving the thoughts that this reaction is initiated by the activation of NMDA receptor it draws attention that NMDA may have impact on human cardiomyocytes. The presence of T1NR in myocardium may suggest that cardiotoxicity of its agonist is due to its prolonged activation by MSG.

Our aim was to investigate the electrophysiological effect of NMDA receptor activation on myocardial action potential.

Methods: All experiments were carried out on enzymatically isolated canine left ventricular cells at 37°C. Sharp microelectrode technique was used to measure action potential (AP). Data is given as average±SEM. Cells were exposed to NMDA (10 or 100 µM) for 8-10 min followed by washout. In some experiments 10 µM NMDA was applied in the presence of the NMDA receptor antagonist, D-AP5 (50 µM).

Results: V+max was reduced in 10 µM NMDA (n=6) and 100 µM NMDA (n=7) by 30.0±9.2% (p=0.022) and 8.3±3.2% (p=0.041), respectively. Phase I amplitude in 10 µM NMDA was also reduced by 55.8±10.2% (p=0.012). V-max, APA, peak potential were also significantly reduced by 100 µM NMDA. These actions of NMDA were hardly reversible upon washout of the drug. No significant change of action potential parameters were found in exposure to D-AP5 or NMDA in the presence of D-AP5 (n=6).

Despite the statistically significant but sometime small effects the small number of sample size limits the evaluation of NMDA activation on cardiac AP.

Conclusion: we cannot rule out that T1NR activation can be responsible for Chinese restaurant syndrome.

Supervisor(s): Norbert Szentandrássy

P6

Apolka Domokos (2nd year PhD student)

Research Center for Molecular Medicine

ANALYSIS OF COW AND HUMAN MILK IMMUNOGLOBULIN N-GLYCANS BY CE-LIF

Milk produced by the mammary glands serves not only as nutrition, but it contains a great number of important signaling molecules and immunoglobulins for neonates both in the animal kingdom as well as in the humans. Immunoglobulin preparations from bovine and human sources have been used in the food industry to ease gastrointestinal infections, and also utilized to fortify baby formulas as a substitute for breastfeeding. It has been recently shown that the immune protein content of human milk is very specific for the needs of newborns, personalized by micro and macro-environmental factors of the mother. It has also been reported that diet-derived IgG could have an impact on the human innate immune system responses in a much longer-term as the first 6 months.

In this study, IgG and IgA molecules were captured from the milk samples by protein G and IgA specific Z-domain variant Ni-IMAC microcolumns. The N-glycans from the captured immunoglobulins were released by PNGaseF digestion, labeled with a charged fluorophore (APTS) and purified by magnetic beads. Analysis of the labeled glycans was carried out by automated capillary electrophoresis with high sensitivity laser-induced fluorescent detection.

First, we have comparatively analyzed the IgG and IgA N-glycan traces of the human milk samples and found significant differences in their profiles. Furthermore, healthy organic and non-organic as well as Endometritis infected cow colostrums were investigated using the same workflow. Alterations in the N-glycan structures were found between healthy organic and non-organic as well as Endometritis infected cow colostrum immunoglobulin samples. These differences could be the consequence of the different antibiotic or other treatment of the animals and should be considered when used in baby formulas.

Supervisor(s): András Guttman

P7

Lilla Egeresi (1st year PhD student)

Department of Medical Imaging

COMPARISON OF CT IMAGE QUALITY AND DOSE FROM ROUTINE ABDOMEN PROTOCOLS AT DIFFERENT SCANNERS

Aims: The automatic exposure control (AEC) depends on the manufacturers, thus the patient related effective dose (EffDose) and image noise may vary across scanner models. At our radiology department there are three different CT scanners in which we intend to standardize the clinical protocols regarding image quality (IQ). The main purpose of this study is to compare radiation dose

and IQ of abdominal CT investigations on the three scanners using the original AEC settings.

Methods: Abdominal CT scans were performed on 422, 451 and 655 patients with Siemens SOMATOM Definition AS (CT3), GE BrightSpeed S (CT2) and GE LightSpeed VCT (CT1), respectively. Circular ROIs with an area of 150 mm2 were manually drawn on single axial slices to obtain the mean and standard deviation (SD) of Hounsfield values in the ROI of the liver, aorta, and muscle for each

scans. The EffDose values were calculated based on DLP, patient weight and body part investigated. The (SD 2 *EffDose) -1 as a figure of merit (FOM) was also calculated.

Results: The noise indexes (SD and SD/mean) and EffDose were inversely changed across the scanner (Fig.1 and Fig.2). The AEC settings produced similar image noise up to 100 kg body weight, but higher noise was observed in greater weights at CT2 and CT3 systems (Fig3). The CT3 has the largest FOM

(Fig. 4).

Conclusion: The three scanners do not produce the same IQ and EffDose at the original AEC settings.

Supervisor(s): László Balkay

P8

Anna Farkas (2nd year PhD student)

Research Center for Molecular Medicine

MODELING OF THE HUMAN SERUM N-GLYCOME BASED ON THE SUPERIMPOSED GLYCOSYLATION PROFILES OF FIVE HIGH ABUNDANT GLYCOPROTEINS

The human serum proteome is an abundant source of potential biomarkers for various diseases. N-glycosylation is one of the most frequent co-and posttranslational modification of serum proteins in eukaryotic cells, which can influence several biological properties of the host proteins including stability, signal transduction, motility, enzyme activity, half-life time. The structures of N-glycans bound to the asparagine residue of glycoproteins at the consensus sequence of Asn-X-Thr/Ser (X cannot be Pro) motif is a result of numerous cell and tissue specific glycosyltransferases and glycosydases, which can create disease specific glycan structures. The most abundant serum glycoproteins in human serum are IgG, IgA, transferrin, haptoglobin and a1- antitrypsin, representing 85% of serum glycoproteins. In this study, we have first analyzed the N-glycosylation profiles of these five high abundant human serum glycoproteins using capillary electrophoresis with laser-induced fluorescent detection (CE-LIF). The total serum N-glycome profile was then modeled by mixing the five in their respective ratios. By careful comparison of the CE-LIF glycosylation trace of patient samples to this model human serum N-glycan profile, disease specific changes could be readily detected without any bias and no requirement for immunoprecipitation or other expensive and time consuming depletion methods.

Supervisor(s): András Guttman

P9

Csenge Filep (1st year PhD student)

Research Center for Molecular Medicine

ANALYSIS OF APTS LABELED PARTITIONED N-LINKED GLYCAN LIBRARIES BY MULTICAPILLARY GEL ELECTROPHORESIS

Analysis of APTS labeled partitioned N-linked glycan libraries by multicapillary gel electrophoresis

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One of the fastest growing biotherapeutic products are glycoproteins. Their glycosylation pattern plays a key role in the biological activities of glycobiotherapeutics, e.g., the effector function, the anti-inflammatory properties and serum half-life can all be affected by the glycosylation changes at the conserved Asn297 site of the CH2 domain of the heavy chain. Thus, there is an increasing need for reproducible high throughput glycoanalytical methods to monitor and characterize the N-glycosylation of therapeutic glycoproteins In this poster we report on the simultaneous analysis of nine APTS labeled partitioned N-linked glycan libraries including high mannose, afucosyl biantennary, fucosyl biantennary and six sialylated types using multicapillary gel electrophoresis. This analysis made possible the generation of a new glucose unit (GU) database, specific for the multicapillary electrophoresis system used. The database contains most of the structures that are present in therapeutic glycoproteins. Using this new database, the N-glycan structures of two glycosylated therapeutic proteins – adalimumab and etanercept – was identified.

Supervisor(s): András Guttman

P10

Marvi Ghani (1st year PhD student)

Department of Medical Chemistry

INVESTIGATING THE ROLE OF PHOSPHORYLATED DRP1 IN THE NEURODEGENERATIVE HUNTINGTON'S DISEASE.

Huntington's disease (HD), a neurodegenerative disorder is related to impaired mitochondrial function due to mutant Huntington protein (Htt) and excessive production of dynamin-related protein (Drp1). In stressed condition, elevation in the fragmented state of mitochondria occurs due to release of nuclear cyclin c which reacts with the GTPase domain of Drp1 and induction of mitochondrial fission occurs. Depletion or dephosphorylation of Drp1 can enhance the immoderate fission of the mitochondria. In neuronal cells of Huntington's disease, the interaction of Drp1 mutants with aggregates of toxic Htt leads to change in mitochondrial morphology, size, and dynamics and ATP production. The current investigation was done to find out the relation between toxic Htt aggregates with the mutant Drp1 protein and the effect of Drp1 depletion (shDrp1 cell line) upon the overexpression of mutant Htt. It was postulated that the aggregates of the mutant Htt decreases in case of the depletion of Drp1. For this study, using control and shDrp1 SH-SY5Y human neuroblastoma cell line, we identified the reduction of mutant Htt aggregates in shDrp1 cell line model. Additionally, we generated several point mutants of Drp1 which include mutations of specific phosphorylation sites. Our main goal is to explore the role of Drp1 phosphorylation in mitochondrial structural changes and activity and on toxic Htt aggregate formation in an in vitro cell model of HD.

Supervisor(s): Krisztina Tar

P11

Eliza Guti (2nd year PhD student)

Department of Medical Chemistry

PARP-1-DEPENDENT LIPID ACCUMULATION IN A BREAST CANCER CELL LINE

Background: Lipid droplets (LDs) are intracellular storage organelles for neutral lipids (NL). The NL core is surrounded by a phospholipid monolayer which is covered with various proteins. Initially, LDs were viewed as passive structures, but later some studies highlighted new roles as well. Tumor development can be characterised by well-defined hallmarks that LDs may influence. Poly(ADP-ribose) polymerase-1 (PARP-1) is a nuclear enzyme that PARylates proteins wich regulate many biological processes including metabolism. In the hypoxic tumor microenvironment, tumor cells adapt by activating HIF-1. Previous studies showed that PARP-1 acts as a transcriptional co-activator of HIF-1 and that LD accumulation contributes to the hypoxia tolerance of breast cancer cells. Despite these studies, many questions are still unclear about the LD biogenesis in breast cancer. Our aim was to examine the relationship between PARP-1 and LD biogenesis in breast cancer cells.

Methods: The genetic modification of JIMT1 breast cancer cell line was created with CRISPR-Cas9 system. The specific inactivation of the parp-1 gene was verified by qPCR and Western blot. LD accumulation was induced by the iron chelator, deferoxamine (DFO) treatment and was examined with Nile Red staining. The cell number was determined by DAPI staining The analysis of parp-1, LD-associated and lipid metabolic gene expression was done by qPCR.

Results: After the transfection of our breast cancer cells with parp-1 gene specific guide RNAs, the gene expression dramatically decreased and the protein was not detectable at all. Due to the DFO treatment, PARP-1 KO cells accumulated more LDs than the WT ones. The parp-1 expression significantly decreased in the WT cells upon DFO treatment. The mtor, fasn, hif1a, ppara and ppar?1 expression was reduced while the adrp and ppard expression increased in both the WT and KO cells after DFO treatment.

Summary: PARP-1 may limit LD accumulation in breast cancer cells associated with reducing cell death or enhancing cell proliferation. Based on the qPCR results, the origin of the LD accumulation was not the higher fatty acid synthesis but the decreased FAO and increased uptake.

Supervisor(s): Zsuzsanna Polgár

P12

Tamás Hézso (1st year PhD student)

Department of Physiology

LATE SODIUM CURRENT IN CANINE, GUINEA PIG AND HUMAN VENTRICULAR MYOCARDIUM

Aims: Although late sodium current (INa-late) has long been known to contribute to plateau formation of mammalian cardiac action potentials, lately it was considered as possible target for antiarrhythmic drugs. However, many aspects of this current is still poorly understood. The present work was designed to study the true profile of INa-late in canine and guinea pig ventricular cells and compare them to INa-late recorded in undiseased human hearts.

Methods: INa-late was defined as a tetrodotoxin-sensitive current, recorded under action potential voltage clamp conditions using either canonic- or self-action potentials as command signals. INa-late was also recorded using conventional voltage clamp.

Results: Under action potential voltage clamp conditions the amplitude of canine and human INa-late monotonically decreased during the plateau (decrescendo-profile), in contrast to guinea pig, where its amplitude increased during the plateau (crescendo profile). The decrescendo-profile of canine INa-late could not be converted to a crescendo-morphology by application of isoproterenol, calmodulin, ramp-like command voltages or command action potentials recorded from guinea pig cells. Conventional voltage clamp experiments revealed that the crescendo INa-late profile in guinea pig is due to the slow decay of INa-late in this species. INa-late was increased by isoproterenol but not by calmodulin in canine myocytes. When action potentials were recorded from multicellular ventricular preparations with sharp microelectrode, tetrodotoxin decreased APD in a reverse rate-dependent manner, which effect was the largest in human, while smaller in canine and the smallest in guinea pig preparations.

Conclusions: It can be concluded that important interspecies differences exist in the expression and behavior of INa-late. Canine myocytes represent a better model of human ventricular cells than those of the guinea pig regarding the properties of INa-late. Accordingly, canine ventricular tissues or myocytes are suggested for pharmacological studies with INa-late modifiers.

Supervisor(s): Balázs Horváth, Péter Pál Nánási, Norbert Szentandrássy

P13

Nikolett Király (2nd year PhD student)

Department of Medical Chemistry

ANNEXIN A2 IS A NOVEL INTERACTING PARTNER OF TIMAP-PP1C COMPLEX IN ENDOTHELIAL CELLS

TIMAP, TGF-beta-inhibited membrane-associated protein is highly expressed in endothelial cells, where it acts as a regulatory subunit of protein phosphatase 1 (PP1). TIMAP contains a potential nuclear localization signal and a prenylation CAAX-box motif, accordingly, it was found in the nucleus and in the plasma membrane of pulmonary artery endothelial cells. It plays an important role in the regulation of the endothelial barrier maintenance through the dephosphorylation of its substrate proteins.

Our recent aim was to identify new interacting partners or substrates of TIMAP-PP1c in endothelial cells. Bacterially expressed GST-tagged full length and truncated TIMAP forms were used in pull down assay made with bovine pulmonary artery endothelial cells (BPAEC). Annexin A2 was identified as an interacting partner of TIMAP by LC-MS/MS analysis. The interaction was confirmed by Western blot and immunoprecipitation. We found that annexin A2 binds to the N-terminal region of TIMAP. To further verify the interaction, recombinant GST-annexin A2 was utilized in additional pull down experiment. It was able to bind TIMAP-PP1c, furthermore, the interaction was more pronounced in the presence of Ca2+ (0.5mM and 1mM). Results of immunofluorescence staining and subcellular fractionations showed mainly nuclear localization of annexin A2. For further studies of annexin A2 in BPAEC, the recombinant plasmid for mammalian expression was prepared by specific primers. Using a C-terminal tag expressing plasmid, the overexpression of recombinant annexin A2 was unsuccessful. With a plasmid, which expresses an N-terminal tagged recombinant, the localization of the fusion protein was different from the endogenous annexin A2, most probably because the N-terminal region of annexin A2 contains several phosphorylation sites, such as Ser11, Ser25 and Tyr23. Subcellular fractionation and confocal micrscopy after immunofluorescent staining of BPAEC showed that Ser25-phosphorylated annexin A2 localized mainly in the nucleus.

In the future, we would like to study the physiological significance of TIMAP-annexin A2 interaction in BPAEC.

Supervisor(s): Anita Boratkó

P14

Zsuzsanna Édua Magyar (1st year PhD student)

Department of Physiology

INVESTIGATION OF RYANODINE RECEPTOR USING PEPTIDE TOXINS

Ryanodine receptor (RyR) is a ligand-gated Ca2+ release channel of the sarco-endoplasmic reticulum. We have shown previously that the scorpion toxin, maurocalcin (MCA) binds to the RyR's pore and locks the channel in a half-open state, which enhances the ion current through the RyR. During our effort to find RyR inhibitors we were looking for pore blocking toxins which shared homologue structure with the MCA and found charybdotoxin (CHTX), a well-known potassium channel blocker to be a potential good candidate. CHTX is a 37 amino acids peptide toxin whose tertiary structure is stabilized by three disulfide bridges so that the positively charged arginine and lysine amino acids are positioned on the surface of the toxin. We tested the effect of CHTX on RyR in single-channel current experiments and found that CHTX induced closed states at 40 nM concentration in an extremely voltage dependent manner. It is known that 27th amino acid of CHTX (a lysine) is responsible for the pore block in K+-channels therefore, we first tested a modified CHTX which contains asparagine instead of lysine in the 27th position of the primary sequence (K27N mutant). We show that the K27N CHTX retained its effect on RyR suggesting that the mechanism of CHTX action is different in the two types of channels.

We used the 3D structures of the RyR and CHTX and performed molecule modelling to find the amino acid which is essential for the pore block in RyR. The modelling predicted three options: the 11th lysine, the 18th lysine and the 34th arginine. Next, K11, K18, R34 were replaced to asparagine and glutamine, respectively and their effects were tested. K11N and K18N showed similar effects as the K27N mutant and the wt, however the R34Q proved to be mostly ineffective. These results show that CHTX blocks the RyR's pore via the 34th arginine.

Supervisor(s): János Almássy

P15

Eszter Maka (1st year PhD student)

Department of Obstetrics and Gynecology

THE IMPORTANCE OF CA125 AND HE4 TUMOUR MARKERS IN THE PREDICTION OF OPERATIVE ELIGIBILITY OF ADVANCED STAGE EPITHELIAN OVARIAN CANCER PATIENTS AND PROGRESSION-FREE SURVIVAL

Introduction: Ovarian cancer is the deadlyest of all gynecological cancers. The most important factor of overall survival is succesful primary debulking surgery (PDS). In advanced stages, the goal is optimal debulking surgery which should be at least >50%, in inoperable cases neoadjuvant chemotherapy may be an option. We aimed to investigate the use of CA125 and HE4 preoperative levels in the prediction of complete tumour reduction during upfront surgery, and the use of postoperative marker levels in the verification of tumour reduction and prediction of progression-free survival (PFS).

Patients and methods: 100 advanced ovarian cancer patients (FIGO III-IV), operated between 2010.01.01-2018.11.30 who had attempted curative primary debulking surgery, had CA125 or HE4 results before and/or after the operation were included. Progression free survival was assessed through Kaplan-Meier analysis, Receiver Operative Characteristics (ROC) curves and area under curve (AUC) was used to evaluate the performance of CA125 and HE4 markers.

Results: Preoperative HE4 levels differed significantly in cases of successful complete and incomplete tumour reduction (median [IQR]: 198,2 [54-573] vs. 836 [184-2912] respectively, $p<0.0001$). Using ROC analysis we determined the AUC, the sensitivity and specificity for CA125 and HE4 in the prediction of complete tumour reduction. Preoperative HE4 proved to be more useful in distinguishing between the two groups. Postoperative HE4 levels also proved to be more helpful than CA125 in the prediction of >1 year progression- free survival.

Conclusion: HE4 proved to be a more sensitive and specific preoperative tool in the selection of patients for later successful complete reduction. Postoperative HE4 levels correlated very strongly with complete tumour reduction, therefore can be used as an independent assurance marker, as well as for the prediction of progression-free survival.

Supervisor(s): Zoard Krasznai

P16

Muhammad Umair Naseem (1st year PhD student)

Department of Biophysics and Cell Biology

PRODUCTION OF AN ION CHANNEL BLOCKER PEPTIDE (MARGATOXIN) IN PICHIA PASTORIS: AN EFFICIENT EUKARYOTIC EXPRESSION SYSTEM

The Kv1.3 voltage gated potassium channels are upregulated during the activation of effector memory T (TEM) cells that is responsible for the chronic inflammation and autoimmune diseases. So, Kv1.3 is an attractive immunomodulatory drug target to treat many autoimmune diseases. Several peptide toxins from animal venom inhibiting Kv1.3 channel with varying affinity and selectivity have been described. Commonly, these disulfide-rich peptides are chemically synthesized which is very expensive and time taking approach. Recombinant production of peptide toxins in bacteria needs additional steps to properly refold the peptide and acquire native disulfide bridging pattern. Thus, we developed and optimized an efficient Pichia pastoris expression system to produce ion channel blocker peptides with better post translation modifications and native disulfide bonds. As an example, we integrated the margatoxin (MgTx) DNA coding sequence into Pichia pastoris X-33 genome and expressed under alcohol oxidase (AOX) promoter. This system secreted 30 mg/L of MgTx which was subsequently, purified by affinity chromatography and HPLC up to 98% purity with 40% recovery. Electrophysiological studies of MgTx against Kv1.3 ion channel of activated human T lymphocytes and Kv1.2 of CHO cells demonstrated that our recombinant MgTx has the comparable blocking effect as of commercially available rMgTx. Hence, this expression system offers an efficient and easy method to produce ion channel blocker peptides. Further, protein engineering and modification of these peptides is underway to achieve high level selectivity and affinity for Kv1.3 ion channel and targeted delivery to the point of action for treatment of autoimmune diseases and cancer.

Supervisor(s): György Panyi

P17

Zsolt Ráduly (1st year PhD student)

Department of Physiology

SEPTIN 7 IS ESSENTIAL FOR THE PROPER DIFFERENTIATION OF C2C12 CELLS

Cytoskeletal components septins have important roles in various cellular processes, e.g. cell mobility, apoptosis, endocytosis and cell shape within a wild range of organisms, including yeast, drosophila and mammals. Different septin isoforms could form hetero-oligomeric complexes like filaments and rings. So far 13 human septins have been described and classified into 4 subgroups, where septin 7 is the only member of its group and it has a crucial role in the formation of higher-order structures of septins.

As the role of septin 7 in skeletal muscle is not fully understood yet, we wanted to examine the effect of septin 7 expression changes on C2C12 cultured cells. We were able to show cytoplasmic distribution of septin 7 in cultured myoblast and myotubes and its co-localization with actin filaments. We tried to generate knockout cell line using the Crispr/Cas9 method, but remaining cells following gene editing were not capable to proliferate. We got only few living cell where the cell division was impaired, so we could identify big, multinucleated cells with disorganized cytoplasm and could not generate septin 7 knockout C2C12 clones. To modify the expression of septin 7 in the cultured cells shRNA technique was applied. However, decreased septin 7 expression generated remarkable changes in cell morphology and other physiological processes. In septin 7 knockdown cells (KD) the average cell area increased compared to both the absolute control and scrambled shRNA transfected cells. The perimeter of septin 7 KD cells did not show any significant alteration, however cells loosed their processes and became more circular than the controls. Cell proliferation and the myotube differentiation were also significantly reduced in KD cells. During the differentiation multinucleated myotubes were hardly detected, the fusion index was markedly reduced, and differentiation marker desmin protein expression was significantly lower in septin 7 modified cultures.

These finding in the skeletal muscle model system C2C12 cells indicate that septin 7 has essential role in the proper development and differentiation process of myotubes.

Supervisor(s): Mónika Dr. Szentandrássyné Dr. Gönczi

P18

Dániel Sárközy (1st year PhD student)

Research Center for Molecular Medicine

ELIMINATING SODIUM DODECYL SULFATE IONIZATION SUPPRESSION IN CAPILLARY GEL ELECTROPHORESIS - ELECTROSPRAY IONIZATION MASS SPECTROMETRY OF PEPTIDES AND PROTEINS

Capillary SDS gel electrophoresis (CE-SDS) is the mostly used routine purity checking method for therapeutic proteins using UV or fluorescent detection. Hyphenation of CE-SDS with electrospray ionization mass spectrometry (ESI-MS) on the other hand, would provide very important structural information. For the time being, this hyphenation is hindered by the presence of the large amount of SDS in the background electrolyte making it non compatible for the electrospray process. Protein solubilization and denaturation prior to CE-SDS necessitates the use of ionic detergents and sodium dodecyl sulfate is one of the most frequently used not only in CE-SDS, but as denaturing agent during tryptic digestion as well. Usual denaturing sample preparation buffers require the use of up to 4% SDS, and CE-SDS gel-buffer systems contain 0.1% SDS (3.5 mM). This surfactant concentration is still too high and has to be reduced prior to entering the mass spectrometer. In positive ionization mode at SDS concentrations >1 mM, strong surfactant related signals (e.g., multimers) override the sample signals, while at lower surfactant levels, e.g., 0.4 mM SDS, ion suppression prevails. To achieve higher protein coverage in ESI-MS after SDS-CGE separation, SDS removal is practically an absolute necessity. In this approach, we utilize the addition of cyclodextrins via the sheath liquid at the moment when the protein-SDS complexes leave the separation capillary column and enter the microreaction chamber with the nanoelectrospray tip. Cyclodextrins (CDs) are cyclic oligosaccharides forming a truncated cone with a hydrophobic internal cavity. The hydrophobic tail of SDS can strongly bind into this cavity resulting in stable inclusion CD-SDS complexes, which do not form positive gas phase ions from charged droplets, thus not introduced into the mass spectrometer. In other words, the formation of positively charged multimers of SDS in the spray is suppressed by the tightly bound CD-SDS inclusion complexes in solution, minimizing the SDS entry to the MS. In this poster, we show the effect of CD to SDS ratio on the signal recovery for peptides and the signal reduction for SDS.

Supervisor(s): András Guttman

P19

László Szabó (1st year PhD student)

Department of Physiology

SEPTIN 7 DOES NOT ALTER EC-COUPLING BUT SEVERLY MODIFIES SKELETAL MUSCLE ARCHITECTURE

Septins are filament-forming cytoskeletal proteins present in several organisms, including yeast, drosophila and mammals. They are referred as the fourth component of the cytoskeleton. Different septin isoforms could form hetero-oligomeric complexes, which have important roles in various cellular processes, e.g. cell mobility, apoptosis, endocytosis and cell shape. According to previous studies, septin 7 is unique out of 13 human septins, because it has a crucial role in the formation of higher-order structures of septins.

The role of septin 7 has not yet been fully understood in skeletal muscle. Our goal was to identify the role of septin 7 in the extinction-contraction coupling, in muscle regeneration and in the formation of the skeletal muscle. Knocking out septin 7 is lethal in embryonic state, so a tissue specific, tamoxifen induced Cre-lox system was used to modify the expression of septin 7 in skeletal muscle. Reduced expression of septin 7 generated no difference in the voltage dependence of EC-coupling and parameters of the calcium transients induced by electric stimuli. Tibialis posterior muscles were prepared for electron microscopic analysis and images from horizontally and transversally orientated samples were taken to study the occurring structural malformations. Decreased septin 7 expression had a high impact on the architecture of skeletal muscle. The individual myofibrils became smaller but their number increased compared to the control mice. The number of mitochondria was also elevated in septin 7 knockdown mice and they formed large mitochondrial networks distorting the already deformed structure. Next we induced skeletal muscle damage in control and septin 7 knockdown mice with $BaCl_2$ injection and septin 7 expression was followed through the tissue repair. In control mice septin 7 expression significantly increased after the muscle injury and by the end of the regeneration its expression went back to normal. In the case of septin 7 knockdown mice the protein expression did not show significant changes.

These novel insights suggest that septin 7 has a crucial role in skeletal muscle formation and regeneration but does not alter EC-coupling.

Supervisor(s): Beatrix Dienes

P20

Vince Szegeczki (1st year PhD student)

Department of Anatomy, Histology and Embryology

ALTERED NEUROPEPTIDE SIGNALING IN TESTIS OF ALZHEIMER'S DISEASE MODELLING MICE

Pituitary adenylate cyclase activating polypeptide (PACAP) is a 38 amino acid neuropeptide which is detectable in the CNS and various peripheral organs. PACAP is ligand of G protein coupled PAC1, VPAC1 and VPAC2 receptors and induces the activity of adenylate cyclase. Therefore, expression of various genes happens by activation of PKA and transcription factors. In peripheral organs, the highest concentration of PACAP was detected in testis. The peptide plays important role in differentiation and maturation of male gonad and it is essential in male reproduction by supporting spermatogenesis. Fertility can be harmed by lack of PACAP.

Alzheimer's disease is the most common cause of dementia in elderly humans. It has been demonstrated that expression of PACAP decreases in Alzheimer's disease. On the other hand, the neuropeptide has important protective effect against the progression of dementia. Similarly to the brain, pathologic amyloid protein can be formed and accumulated in the testis in Alzheimer's disease.

The main goal of our research was to examine PACAP signalling in testis of Alzheimer's disease modelling mice. We compared the testicles of wild type and PDAPP transgenic mice by performing immunohistochemistry staining, RT-PCR and Western blot analysis.

Decreased expression of PAC1 receptor, phosphorylated PKA, PP2A and phosphorylated Sox9 was detected in testes reflecting on reduced activity of PACAP signalling. Another notable observation was the abnormal collagen type IV content of basal membrane in seminiferous tubules of PDAPP mice compared with that of the testis of wild type mice.

Based on our results and previous investigations it may be hypothesized that examination of signalling pathways in testis can be appropriate for modelling pathologic conditions of CNS in Alzheimer's disease.

Supervisor(s): Tamás Juhász

P21

Zsófia Thalwieser (1st year PhD student)

Department of Medical Chemistry

ANGIOGENIC PROPERTIES OF PROTEIN PHOSPHATASE 2A

Protein phosphatase 2A (PP2A) is one of the main phospho-serine/threonine specific protein phosphatases in mammalian cells. The PP2A holoenzyme consists of a scaffold A subunit, a catalytic C subunit and one of the regulatory B subunits which are assigned into four structurally different families (B, B', B", B'"). The variable B subunits determine substrate specificity and/or subcellular localization of the PP2A holoenzyme. PP2A has an important role in the regulation of the cell cycle, cell differentiation, signal transduction, cytoskeletal remodeling and even cellular dysfunction. Previously our workgroup has shown the role of B55a regulatory subunit in endothelial cell junction regulation.

Our recent aim was to study the role of the PP2A B55a holoenzyme in angiogenesis. NonsiRNA and B55a specific siRNA treated cell lysates and supernatants were studied by Proteome Profiler Angiogenesis Array. The array detects 55 angiogenesis related protein levels simultaneously. We observed that due to B55a depletion, thrombospondin-1 (TSP1) level of the endothelial cells decreased greatly. TSP1 is known as an angiogenesis inhibitory protein by regulating cell migration. Interestingly, not only the protein level, but also the mRNA level of TSP1 decreased in the B55a silenced cells. Furhermore, the tube formation ability of the B55a depleted cells increased compared to the control and nonsiRNA treated cells.

In endothelial cells, the interaction between B55a and TSP1 was proved by immunoprecipitation and also by pull down assay. The TSP1 protein interacted only with B55a isoform of the PP2A B subunit family. Various treatments have shown that TSP1 is phosphorylated by protein kinase C on a serine side chain. Next, regulatory role of B55a on TSP1 degradation will be tested. Also, wild type and PKC site mutant recombinant TSP1 proteins will be created.

Supervisor(s): Anita Boratkó

P22

Gyula Ujlaki (1st year PhD student)

Department of Medical Chemistry

THE CONNECTION BETWEEN THE HUMAN MICROBIOME AND NEOPLASTIC CHANGES IN BREAST TISSUES.

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The gut microbiome is the largest bacterial population in the human body. Its composition depends on the the immune system, the quality of ingested food and xenobiotics. Changes to the composition of the microbiome and the microbial metabolome receives high attention in life sciences.

Our research group investigates the effects of microbial metabolites in neoplastic dieases, in which the tumor is not in direct contact with the gut microbiome. Such neoplasm is breast cancer, which is the most frequent cancer type in women. We aimed for a high throughput assessment of changes in microbiome metabolism in breast cancer. Our investigation had two arms, an in vitro screening of metabolites and a metatranscriptome sequencing arm. For in vitro experiments, we used MCF7 human, and 4T1 mouse breast cancer cell lines. We treated these cell lines with 33 bacterial metabolites in serum physiological concentrations and after 48 hours, we stained them with TexasRed Phalloidin and DAPI to mark the actin filaments and nuclei. Using high content screening and image analysis techniques, we analyzed the morphology and proliferation changes in the cell cultures. From these bacterial metabolites, we successfully identified 9, which shifted both the MCF7 and 4T1 cell line morphology toward mesenchymal and decreased cell proliferation. Another 12 of those metabolites shifted the morphology in both cell lines toward epithelial and increased cell proliferation. Our current results show that there is a complex connection between gut microbiome and breast cancer aggressiveness.

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Supervisor(s): Péter Bay

P23

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Department of Physiology

CALCIUM-ACTIVATED CHLORIDE CURRENT IS ANTIARRHYTHMIC BY REDUCING BOTH SPATIAL AND TEMPORAL HETEROGENEITY OF CARDIAC REPOLARIZATION

The role of Ca2+-activated Cl- current (ICl(Ca)) in cardiac arrhythmias is still controversial. It can generate delayed afterdepolarizations in Ca2+-overloaded cells while in other studies incidence of early afterdepolarization (EAD) was reduced by ICl(Ca). Therefore our goal was to examine the role of ICl(Ca) in spatial and temporal heterogeneity of cardiac repolarization and EAD formation.

Experiments were performed on isolated canine cardiomyocytes originating from various regions of the left ventricle; subepicardial, midmyocardial and subendocardial cells, as well as apical and basal cells of the midmyocardium. ICl(Ca) was blocked by 0.5 mmol/L 9-anthracene carboxylic acid (9-AC). Action potential (AP) changes were tested with sharp microelectrode recording. Whole-cell 9-AC-sensitive current was measured with either square pulse voltage-clamp or AP voltage-clamp (APVC). Protein expression of TMEM16A and Bestrophin-3, ion channel proteins mediating ICl(Ca), was detected by Western blot.

9-AC reduced phase-1 repolarization in every tested cell. 9-AC also increased AP duration in a reverse rate-dependent manner in all cell types except for subepicardial cells. Neither ICl(Ca) density recorded with square pulses nor the normalized expressions of TMEM16A and Bestrophin-3 proteins differed significantly among the examined groups of cells. The early outward component of ICl(Ca) was significantly larger in subepicardial than in subendocardial cells in APVC setting. Applying a typical subepicardial AP as a command pulse resulted in a significantly larger early outward component in both subepicardial and subendocardial cells, compared to experiments when a typical subendocardial AP was applied.

Inhibiting ICl(Ca) by 9-AC generated EADs at low stimulation rates and their incidence increased upon beta-adrenergic stimulation. 9-AC increased the short-term variability of repolarization also.

We suggest a protective role for ICl(Ca) against risk of arrhythmias by reducing spatial and temporal heterogeneity of cardiac repolarization and EAD formation.

Supervisor(s): Balázs Horváth

P24

Florina Zákány (3rd year PhD student)

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CHARACTERIZATION OF DIRECT CYCLODEXTRIN EFFECTS ON KV1.3 ION CHANNEL

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six (alpha CD), seven (beta CD) or eight (gamma CD) alpha-D-glucopyranoside units. Due to their truncated cone structure with an interior hydrophobic cavity they can form complexes with various drugs and lipid molecules. CDss are widely used as inert carriers and in research to selectively deplete cholesterol levels in biological membranes (as methyl-beta-ciklodextrin; MBCD). The direct effects of CDs on ion channels have not been studied yet. Since voltage gated potassium (Kv) channels regulate a great variety of biological processes, direct effects of CDs on these channels can contribute to the known side effects (such as immunsupression via Kv1.3) of numerous drugs.

Our aim was to characterize direct ligand effects of MBCD and three inverted cyclodextrins (with hydrophilic surface and hydrophobic interior made of six, seven or eight 3,6-anhydro-glucose, namely ialpha CD, ibeta CD and igamma CD, respectively) on Kv1.3 and to demonstrate that these are independent from their effects on cholesterol depletion.

We examined direct effects on CHO cells transfected with Kv1.3 ion channels and EGFP using patch clamp technique. CDs at concentrations of 1 and 5 mM partially reversibly inhibited Kv1.3 currents within 15 seconds, except for ibeta CD, which had no such effect. After an incubation period of 1 hour, we measured the extent of cholesterol depletion using a colorimetric kit. Only MBCD resulted in significant cholesterol extraction, while iCDs exerted no effects on cholesterol levels.

Based on our results we can conclude that CDs exert previously unknown direct inhibitory effects on Kv ion channels independent from cholesterol depletion, which can play an important role in side effects of drugs containing CDs.

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Supervisor(s): György Panyi

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