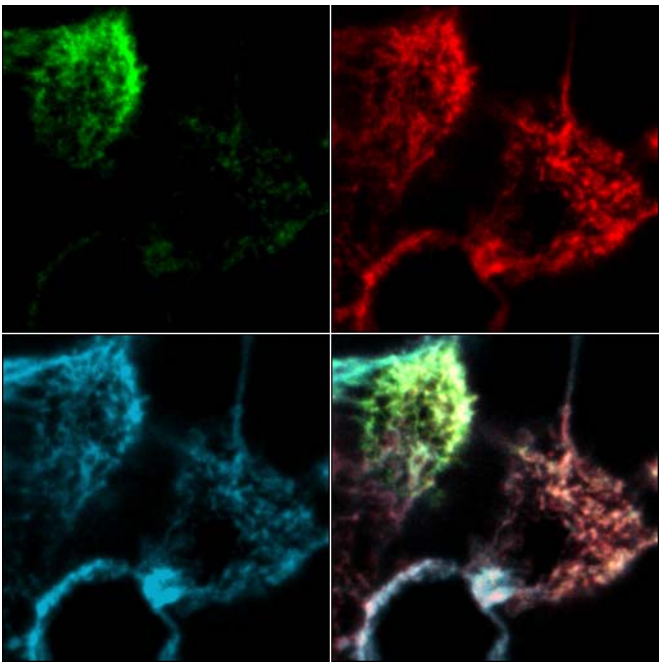
ANNUAL SYMPOSIUM OF THE DOCTORAL SCHOOL OF MOLECULAR MEDICINE, UNIVERSITY OF DEBRECEN



JUNE 6-7, 2011

Cover page:
Co-localization of HLA-DR (green), CD44 (red) and HLA I (blue) on OCM-3 human uveal melanoma cells, as detected by CLSM. Proteins were targeted by Cy2-L243, XTRITC-MEM85, and Cy5-W6/32 mAbs, respectively. Membrane areas where two or three membrane species co-localize are indicated with mixed colors in the overlay image. The applied colors are pseudocolors.

Annual Symposium of the Doctoral School of Molecular Medicine

University of Debrecen
June 6-7, 2011

Structure of the Doctoral School of Molecular Medicine

Head of the Doctoral School	Secretary of the Doctoral School
László Csernoch DSc, full professor Head of the Department of Physiology, Medical and Health Science Center, University of Debrecen	**Andrea Dóczy-Bodnár** PhD, senior research fellow Cell Biology and Signaling Research Group of the Hungarian Academy of Sciences, Department of Biophysics and Cell Biology, Medical and Health Science Center, University of Debrecen

Doctoral Programs

	Head	Core members	Members
I. Cell and molecular biology of signal transduction	**László Virág**	Csilla Csortos Viktor Dombrádi Ferenc Erdődi Pál Gergely	Péter Bai Éva Bakó Ilona Farkas Zsigmond Fehér András Guttman György Haskó Endre Kókai Beáta Lontay András Penyige Gábor Somogyi Éva Szabó György Vargha Róza Zákány
II. Membrane biophysical questions and research methods	**János Szöllősi**	Sándor Damjanovich Zoltán Krasznai Teréz Márián László Mátyus Péter Nagy György Panyi	László Balkay László Bene Andrea Dóczy-Bodnár Miklós Emri Zsolt Fazekas Péter Hajdú Attila Jenei Lajos Trón György Vámosi Zoltán Varga Zsuzsa Varga
III. Physiology and neurobiology	**László Csernoch**	Tamás Bányász Tamás Bíró László Galuska László Kovács János Magyar Zoltán Rusznák Géza Szűcs	Ervin Berényi Gabriella Czifra Julianna Cseri István Jóna Sándor Sárközi András Szatmári Norbert Szentandrássy Péter Szentesi

Registered PhD students of the Doctoral School (2nd semester, 2011)

Doctoral program	Name	Supervisor(s)
I. Cell and molecular biology of signal transduction	Bálint Bécsi, 3rd year	Ferenc Erdődi
	András Kovács, 3rd year	András Guttman
	Stefan Mittermayr, 3rd year	András Guttman
	Magdolna Szántó, 3rd year	Péter Bai
	Anita Boratkó, 2nd year	Csilla Csortos
	Éva Kerekes, 2nd year	Endre Kókai
	Petra Lakatos, 2nd year	László Virág
	Adrienn Sipos, 2nd year	Beáta Lontay
	Csilla Somogyi, 2nd year	Róza Zákány
	Ákos Szekrényes, 2nd year	András Guttman
	Éva Katona, 1st year	Róza Zákány
	Zoltán Kónya, 1st year	Ferenc Erdődi
	Roland Ádám Takács, 1st year	Róza Zákány
II. Membrane biophysical questions and research methods	Ádám Bartók, 3rd year	Zoltán Varga
	Dilip Shrestha, 3rd year	Attila Jenei/János Szöllősi
	Tímea Váradi, 3rd year	Péter Nagy
	Julianna Volkó, 3rd year	György Vámosi/László Damjanovich
	Attila Forgács, 2nd year	László Bene
	Imre Lajtos, 2nd year	László Balkay
	Orsolya Szilágyi, 2nd year	Péter Hajdú
	Enikő Várhalminé Németh, 2nd year	Teréz Márián
	István Csomós, 1st year	Andrea Dóczy-Bodnár
	Brigitta Domján, 1st year	Zoltán Krasznai
	Tamás Kovács, 1st year	Péter Nagy
III. Physiology and neurobiology	Dénes Nagy, 3rd year	Géza Szűcs
	Attila Oláh, 3rd year	Tamás Bíró
	Zoltán Palicz, 3rd year	Péter Szentesi
	Olga Ruzsnavszky, 3rd year	László Csernoch
	László Szabó, 3rd year	Géza Szűcs
	Lídia Ambrus, 2nd year	Tamás Bíró
	Nikolett Geyer, 2nd year	László Csernoch
	Erika Lisztes, 2nd year	Tamás Bíró
	Zsuzsanna Nagy, 2nd year	Gabriella Czifra
	Ferenc Ruzsnavszky, 2nd year	János Magyar
	Dóra Bodnár, 1st year	Péter Szentesi
	Angéla Fodor, 1st year	Tamás Bíró
	Kornél Kistamás, 1st year	János Magyar
	Levente Lánczi, 1st year	Ervin Berényi
	Magdolna Szilasi, 1st year	Tamás Bíró

Pre-degree certificates
(June 1, 2010 – May 31, 2011)

Doctoral program	Name	Supervisor(s)
I. Cell and molecular biology of signal transduction	Róbert Bátori Dóra Dedinszki Anita Kása	Ferenc Erdődi Ferenc Erdődi Csilla Csortos
II. Membrane biophysical questions and research methods	Zoltán Fodor Eszter Kosaras Ágnes Tímea Dr. Nagyné Szabó	Teréz Márián Zsuzsa Varga János Szöllősi/Péter Nagy
III. Physiology and neurobiology	István Borbíró Márta Füzi Gábor Harmati Áron Kőszeghy Tamás Oláh Attila Gábor Szöllősi	Tamás Bíró László Csernoch/György Paragh János Magyar Zoltán Rusznák László Csernoch Tamás Bíró

PhD dissertations defended in last year (June 1, 2010 – May 31, 2011)

Sándor Attila Kis (supervisor: Miklós Emri)
Monte-Carlo simulation based analysis of performance parameters in the MiniPET scanner, developed for preclinical studies

László Kovács (supervisor: Viktor Dombrádi)
Examination of Calpain and protein kinase/phosphatase systems – interaction between the post-synthetic protein modifying systems

Pál Pap (supervisor: Géza Szűcs)
Studying the effect of cholinergic modulation on giant cells and astrocytes of the rat cochlear nucleus

Tamás Juhász (supervisor: Róza Zákány)
Optimized transient transfection: an approach to explore the function of signaling proteins regulating chondrogenesis in micromass cell cultures

István Borbíró (supervisor: Tamás Bíró)
New mechanisms in the biological processes of human hair growth regulation

Emese Zsiros (supervisor: György Panyi)
Ion channels in native environment: characterization of ion channels in dendritic and endothelial cells

Nóra Dobrosi (supervisor: Tamás Bíró)
Role of the endocannabinoid system in the regulation of biological processes of human skin derived cells

Ágnes Tímea Dr. Nagyné Szabó (supervisor: János Szöllősi/Péter Nagy) Quantitative description of the constitutive and ligand-induced associations of ErbB receptors

Program of the Symposium

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

Duration of the lectures: 10min + 5min discussion.

June 6, 2011

12:15	**Arrival** (coffe, cake)
12:40	**Welcome address –** László Csernoch
	Moderators **Beáta Lontay** **Péter Szentesi** **Zoltán Varga**
12:45-14:15	**Section I.** *Chairman* **László Csernoch**
	Speakers
12:45-12:55	**Orsolya Szilágyi 2nd year PhD student** supervisor: Péter Hajdú Functional expression of Nav1.7 sodium channels in the $CD1a^+$ subset of monocyte derived dendritic cells
13:00-13:10	**Petra Lakatos 2nd year PhD student** supervisor: László Virág 3-aminobenzamide protects human keratinocytes from UV-induced cell death by a PARP independent mechanism
13:15-13:25	**Éva Katona 1st year PhD student** supervisor: Róza Zákány Identification of potassium cation channels in differentiating chondrocytes
13:30-13:40	**Erika Lisztes 2nd year PhD student** supervisor: Tamás Bíró Investigation of the cannabinoid system on human hair follicle-derived outer root sheath (ORS)

	keratinocytes
13:45-13:55	**Csilla Somogyi 2nd year PhD student** supervisor: Róza Zákány Functional characterisation of Transient Receptor Potential Vanilloid (TRPV) 1 ion channel expression during in vitro chondrogenesis
14:00-14:10	**Dénes Nagy 3rd year PhD student** supervisor: Géza Szűcs Role of the mitochondrial TASK-3 channels in melanoma cells
14:15-14:30	**Coffee break**
14:30-15:45	**Section II.** *Chairman* **László Mátyus** *Speakers*
14:30-14:40	**Dilip Shrestha 3rd year PhD student** supervisor: Attila Jenei/János Szöllősi FRET reveals the association of CD1d with MHC molecules on the surface of a B lymphocyte
14:45-14:55	**Ferenc Ruzsnavszky 2nd year PhD student** supervisor: János Magyar Cardiac electrophysiological effects of rosiglitazone in canine ventricular cells
15:00-15:10	**Anita Boratkó 2nd year PhD student** supervisor: Csilla Csortos PP2A dephosphorylates ezrin-radixin-moesin (ERM) -binding phosphoprotein 50 at the mitotic phase of the cell cycle
15:15-15:25	**Attila Oláh 3rd year PhD student** supervisor: Tamás Bíró Effects of nicotinic acid on the biological functions of human sebocytes
15:30-15:40	**Magdolna Szántó 3rd year PhD student** supervisor: Péter Bai Investigation of the effects of PARP-2 depletion on SIRT1 activation

15:45-16:00	**Coffee break**
16:00-17:15	**Section III.**
	Chairman
	László Virág
	Speakers
16:00-16:10	**Roland Ádám Takács 1st year PhD student** supervisor: Róza Zákány
	Characterisation of the chondrifying high density cultures of the BMP2-producing C3H10T mouse embryonic mesenchymal cell line
16:15-16:25	**Lídia Ambrus 2nd year PhD student** supervisor: Tamás Bíró
	The possible regulatory role of TRPC6 channel in mouse and human immortalized podocytes
16:30-16:40	**Julianna Volkó 3rd year PhD student** supervisor: György Vámosi/László Damjanovich
	MHC I organizes protein clusters and inhibits IL-2/IL-15 signaling in human T cells
16:45-16:55	**Stefan Mittermayr 3rd year PhD student** supervisor: András Guttman
	Multiplexed glycoanalytics: bioinformatics assisted rapid and confident N-Glycan structural elucidation
17:00-17:10	**András Kovács 3rd year PhD student** supervisor: András Guttman
	Fractionation of the human plasma proteome for monoclonal antibody proteomics based biomarker discovery

June 7, 2011

12:00	**Arrival** (coffe, cake)
	Moderators
	Péter Bai **Péter Hajdú** **Norbert Szentandrássy**

12:30-13:45	**Section IV.**
	Chairman
	István Jóna
	Speakers
12:30-12:40	**Adrienn Sipos 2nd year PhD student** supervisor: Beáta Lontay
	Nuclear localization, function and regulation of myosin phosphatase
12:45-12:55	**Nikolett Geyer 2nd year PhD student** supervisor: László Csernoch
	Effects of nifedipine treatment on calcium homeostasis of urinary bladder smooth muscle cells
13:00-13:10	**Zsuzsanna Nagy 2nd year PhD student** supervisor: Gabriella Czifra
	Examination of RasGRP3 expression on human breast cancers and breast-derived ductal adenocarcinoma cell lines
13:15-13:25	**Imre Lajtos 2nd year PhD student** supervisor: László Balkay
	Investigation of the low count detectability in nuclear medicine images using human and model observers
13:30-13:40	**Attila Forgács 2nd year PhD student** supervisor: László Bene
	CD44-ICAM-1 crosstalk alters MHCI rafts
13:45-14:00	**Coffee break**
14:00-15:15	**Section V.**
	Chairman
	Viktor Dombrádi
14:00-14:10	*Speakers*
	Dóra Bodnár 1st year PhD student supervisor: Péter Szentesi
	Effects of selenium on the contractile properties of mouse skeletal muscles
14:15-14:25	**Tímea Váradi 3rd year PhD student** supervisor: Péter Nagy
	Binding of trastuzumab to ErbB2 is inhibited by a

	high local density of hyaluronan
14:30-14:40	**Éva Kerekes 2nd year PhD student** supervisor: Endre Kókai Characterization of R5 glycogen binding subunit homolog in Drosophila
14:45-14:55	**Olga Ruzsnavszky 3rd year PhD student** supervisor: László Csernoch HaCaT keratinocytes exhibit spontaneous intracellular calcium transients
15:00-15:10	**Tamás Oláh Predoctor** supervisor: László Csernoch Trisk 32 regulates IP3 receptors in rat skeletal myoblasts
15:15-15:30	**Coffee break**
15:30-16:30	**Section VI.** *Chairman* **János Szöllősi**
15:30-15:40	**Zoltán Palicz 3rd year PhD student** supervisor: Péter Szentesi In vivo test of a highly stable antifungal protein (PAF) against lung aspergillosis
15:45-15:55	**Ádám Bartók 3rd year PhD student** supervisor: Zoltán Varga Electrophysiology of differentiating chondrocytes
16:00-16:10	**Enikő Nizsalóczki Predoctor** supervisor: Andrea Dóczy-Bodnár/György Vámosi Biophysical investigation of IL-9R assembly and function in human T-lymphoma cells
16:15-16:25	**Bálint Bécsi 3rd year PhD student** supervisor: Ferenc Erdődi Application of SPR technique to characterize the interaction of membrane-permeable enzyme inhibitors with lipid micelles
16:30	**Conclusion** – János Szöllősi

Abstracts

June 6, 2011

Functional expression of Nav1.7 sodium channels in the CD1a$^+$ subset of monocyte derived dendritic cells

Orsolya Szilágyi 2nd year PhD student
Department of Biophysics and Cell Biology
Tutor: Péter Hajdu

Dendritic cells (DCs) function as professional antigen presenting cells and participate in the initiation of adaptive immune response. Human monocyte-derived DCs have two developmental phenotypes: immature DCs (IDCs) take up and process foreign antigens, while mature DCs are able to trigger T cells in the lymph nodes. According to their CD1a expression two subpopulations of DCs are distinguished: CD1a$^+$ DCs differentiate into Langerhans cells in the epidermis and other epithelial surfaces, whereas the CD1a$^-$ subtype replenishes various tissues with interstitial/tissue DCs. We described earlier the expression of Nav1.7 ion channels in IDCs, and here we present its subtype-specific expression and function.

Human DCs were produced from freshly isolated monocytes according to the procedure developed in our laboratories. Expression level of various genes was detected using TaqMan Q-RT-PCR, and was verified with standard whole-cell patch-clamp recording. To measure membrane potential in IDCs current-clamp configuration of patch-clamp technique was applied. Migration experiments of IDCs were performed using standard protocol (Boyden chamber).

The incidence and expression level of Nav1.7 current in CD1a$^+$ IDCs were higher than in the CD1a$^-$'s. Both electrophysiological and molecular biological approaches revealed the similar expression level of IKCa1, a Ca^{2+} activated K^+ channel in two subtypes of IDCs. Membrane potential recording in CD1a$^+$ IDCs exhibited extremely high value (app. -10 mV) compared to the CD1a$^-$'s (app. -60 mV). Upon perfusion with Na^+-free extracellular solution the membrane potential of CD1a$^+$ cells shifted toward negative values (app. -50 mV) unlike in CD1a$^-$ cells. Silencing of the SCN9A gene (coding Nav1.7) shifted the membrane potential to a hyperpolarizing direction in CD1a$^+$ IDC. Both pharmacological inhibition (application of TTX) and down-regulation of Nav1.7 resulted in the decrease of migratory capacity of CD1a$^+$ IDCs. Fine tuning of IDC functions by a voltage-gated sodium channel emerges as a new regulatory mechanism modulating the migration and cytokine responses of these DC subsets.

3-aminobenzamide protects human keratinocytes from UV-induced cell death by a PARP independent mechanism

Petra Lakatos 2nd year PhD student
Department of Medical Chemistry
Tutor: László Virág

Human skin, unlike all other organs is continuously and directly exposed to enviromental influences. Solar UV radiation is divided into three regions depending on wavelength, short-wave UVC (200-280 nm), mid-wave UVB (280-320 nm), and long-wave UVA (320-400 nm) radiation. In particular UVB is known to alter cellular functions via DNA damage, generation of reactive oxygen species (ROS), and the consecutive alterations in a large variety of signaling events.

Poly(ADP-ribosyl)ation (PARylation) is a NAD-dependent protein modification carried out by members of the PARP [poly(ADP-ribose) polymerase] enzyme family. As PARP1 and PARP2 are activated by DNA damage, we set out to investigate the role of PARylation in UV-induced cell death of normal human epidermal keratinocytes. First we determined cell viability of NHEK following irradiation with different doses (0,05-4J/cm^2) of UVB in order to determine the effective doses of UVB in our model. UVB radiation caused a concentration-dependent cell death as measured/assessed with MTT assay, caspase activation, DNA fragmentation and cell morphology (May-Grünwald-Giemsa staining). High doses (2-4J/cm^2) of UVB induced PARP activation as measured by PARP activity assay and PAR immunostaining. The PARP inhibitor 3-aminobenzamide (3-AB) provided significant protection from UVB-induced cell death. However, the more potent and specific PARP inhibitor PJ34 (and several other potent and specific inhibitors) failed to protect cells from UVB, while effectively blocking PAR synthesis in UVB-irradiated cells. These data indicate that 3-AB protects keratinocytes from UVB-induced death by a PARP-independent mechanism.

We are currently characterizing the mechanism of the PARP independent protective effects of 3-AB in UV induced keratinocyte cell death.

Identification of potassium cation channels in differentiating chondrocytes

Éva Katona 1st year PhD student
Department of Anatomy, Histology and Embryology
Tutor: Róza Zákány

In vitro chondrogenesis is characterised by specific ion-concentration changes, however, no data are available concerning either the expression pattern of K^+ channels or their function in adjusting the membrane potential in differentiating chondroprogenitor cells.
The expression pattern and function of K^+ ion channels were investigated in cells of chondrifying high density primary mesenchymal cell cultures established from distal limb buds of 4-day-old chicken embryos.
We detected the mRNA and protein expression of voltage gated potassium channels Kv1.1, Kv1.3 and Kv4.1 during cartilage differentiation. The mRNA expression of KV4.1 followed a peak-like pattern with a considerable elevation on days 2 and 3 compared to day 0, while the mRNA expression pattern of KV1.3 was found to increase towards the end of the differentiation period. To the contrary, the mRNA expression level of KV1.1 was found to be relatively constant during differentiation. The protein expression of all three Kv channels followed very similar pattern when Western analyses were performed on total cell lysates. However, in the plasma membrane fraction, we could only detect the presence of Kv1.1 and Kv4.1 channels; Kv1.1 exhibited a strong expression on culturing days 1 and 2, while Kv4.1 was characterised by a constant low level expression. The role of Kv channels in chondrogenesis was studied by the administration of tetraethylammonium (TEA) to the culture medium. TEA treatments (20 mM) attenuated matrix production, as well as the expression of cartilage-specific marker molecules. While administration of TEA did not interfere with the mitochondrial activity, it significantly reduced the proliferation rate of cells in HDC. According to patch clamp measurements, two-pore potassium channels (K2P) might also contribute to the hyperpolarisation and indeed, mRNAs of seven members of K2P channel-family were also found to be expressed by cells of HDC.
Consequently, Kv channels – presumably with the K2p channels – may play a role in the regulation of the proliferation and differentiation of chondrogenic cells via adjustment of the resting membrane potential.
Support: ETT 022/09 and OTKA-CNK 80709

Investigation of cannabinoid system on human hair follicle-derived outer root sheath (ORS) keratinocytes

Erika Lisztes 2nd year PhD student
Department of Physiology
Tutor: Tamás Bíró

We have previously shown that both the prototypic endocannabinoid anandamide (AEA) – known to be produced by human hair follicles (HFs) – and the exocannabionoid delta9-tetrahydrocannabinol (THC) inhibited hair shaft elongation, proliferation and induced apoptosis-driven catagen transformation of HFs in vitro, by activating cannabionoid receptor-1 (CB1). In the current study we have investigated the presence of the endocannabinoid system (ECS) and the effects of two pharmaceutical patent pending non-psychoactive phytocannabinoid (C1 and C2) on human HF derived outer root sheath (ORS) keratinocytes.

Similar to HFs, we confirmed the expression of CB1 on ORS keratinocytes. Using quantitative real-time PCR, we also identified transient receptor potential (TRP) channels (TRPV1, TRPV2, TRPV4) which are known to be modulated by cannabinoid substances. Moreover, we have also demonstrated the expression of enzymes catalyzing endocannabinoid synthesis (NAPE-PLD, DAG-lipase alpha and beta) and metabolism (FAAH, MAG-lipase). These results suggest that ORS keratinocytes possess a complex ECS.

To investigate the functional role of the ECS and the effect of phytocannabinoids on the basic cellular functions of ORS keratinocytes, cells were treated with AEA, C1 and C2. All cannabinoids significantly decreased cellular proliferation (MTT assay), and markedly suppressed the mitochondrial membrane potential (DilC1(5) staining), which is an early hallmark of apoptosis. Furthermore we found that even the highest concentration (50 μM) of cannabinoids did not induce cytotoxicity (SYTOX Green staining). In our current experiments we are investigating the influence of cannabinoids on the differentiation (cytokeratin profile) and immunological functions (cytokine and antimicrobial peptide production) of the ORS keratinocytes.

In our further studies, we aim to identify the molecular target(s) of the above cannabinoid substances using pharmacological inhibition and RNAi based silencing of the potential receptors to better understand the role of ECS in hair growth and inflammatory skin disorders, such as acne vulgaris which is characterized by hyperkeratinization of HF duct.

Functional characterisation of Transient Receptor Potential Vanilloid (TRPV) 1 ion channel expression during *in vitro* chondrogenesis

Csilla Somogyi 2nd year PhD student
Department of Anatomy, Histology and Embryology
Tutor: Róza Zákány

Mutations in the nonselective cation channel TRPV4 plasma membrane receptors are implicated to be responsible for certain types of skeletal dysplasia and mature articular chondrocytes have been reported to express it. However, little is known about the involvement and function of other TRPV ion channels in cartilage formation.
Our aim was to identify and study the role of the capsaicin-sensitive TRPV1 receptor in differentiating cartilage. Chondrifying high density cell cultures (HDC) were used as experimental models; chondrogenic cells were either isolated from limb buds of chicken embryos, or as a mammalian model, cells of the murine embryonic mesenchymal C3H10T1/2 cell line were used. While HDC established from chicken mesenchymal cells undergo spontaneous chondrogenesis, cells of the murine mesenchymal cell line were transfected with BMP2, which enables differentiation primarily towards the chondrogenic lineage. The mRNA and protein expression of TRPV1 were studied by RT-PCR and Western blot analyses, respectively. The TRPV1 receptor agonist capsaicin and resiniferatoxin (RTX), or the antagonist capsazepine (CZ) were administered to HDC on various days of culturing to study the function of the receptor. As a natural activating factor high temperature (41 and 45°C) or acidic pH (pH 5) were also applied.
The mRNA expression of TRPV1 in chicken and murine HDC showed a characteristic and time-dependent expression pattern. Treatments with TRPV1 agonists and antagonist in chicken HDC resulted in controversial data. However, administration of the agonists in the murine model considerably increased the amount of cartilage matrix production. Low pH and high temperature enhanced the amount of metachromatic matrix areas in both models. The effects of TRPV activation on the expression of cartilage specific genes, cell proliferation and viability were also investigated.
The fact that the capsaicin-binding region of chicken TRPV1 is rudimentary might be accounted for the observed differences in the two models. Our results imply that TRPV1 channels may play a positive role during in vitro chondrogenesis.
Our work was supported by grants ETT 022/09 and OTKA-CNK 80709.

Role of the mitochondrial TASK-3 channels in melanoma cells

Dénes Nagy 3rd year PhD student
Department of Physiology
Tutor: Géza Szűcs

It has been previously described by our research group that melanoma cells showed increased TASK-3 expression. Consequently, for studying the mitochondrial function of the channel protein, a stably gene-silenced cell line was created using RNA interference in the primary tumour-related, immortalised WM35 melanoma cell line. Scrambled control was also applied. Based on previous immunocytochemical experiments, the channel protein shows heavy mitochondrial localisation. According to our hypothesis, the mitochondrial TASK-3 exert antiapoptotic effects via maintaining mitochondrial membrane potential.
The mitochondrial localization of the TASK-3 channels was further confirmed using a sucrose gradient-based separation method. The qualitative determination of the channel protein was carried out in Western blot experiments. The presence of the mitochondrial membrane fragments in the various fractions was verified on the basis of their succinate dehydrogenase subunit A content. Our hypothesis was supported by the finding that these fractions contained TASK-3 channels as well. To exclude the possibility that cell membrane fragments contaminated the investigated fractions, the P2X7 purinergic receptor immunpositivity was tested.
To monitor mitochondrial function, the acetoxy-methyl ester form of the RHOD 123 fluorescent dye was applied in a concentration of 10 μmol/l. The release of the dye from the mitochondria on their depolarization, however, increases the cytosolic flourescence intensity level. The carbonyl cyanide m-chlorophenyl hydrazone was used at micromolar concentrations to evoke mitochondrial depolarization. The experiments revealed significant differences between the responses of the knock-down and the other cell lines, respectively. These differences were seen both in the ratio of the responding cells and in the magnitude of the response. The data indicated that the TASK-3 knock-down cells likely had decreased mitochondrial membrane potential, and that their membrane potential could be decreased more easily. The MTT assay, that measures the mitochondrial reducing capacity, also indicated reduced mitochondrial function in the knock-down cell cultures.

FRET reveals the association of CD1d with MHC molecules on the surface of a B lymphocyte

Dilip Shrestha 3rd year PhD student
Department of Biophysics and Cell Biology
Tutor: Attila Jenei / János Szöllősi

Cluster of Differentiation 1 (CD1) represents the family of proteins that has gained recognition for its important roles in innate and adaptive immunity. While lots have been heard about the peptides, serving as an antigen through MHC molecules, in the activation of T cells; similar roles of lipids came to the foresight only with the discovery of these CD1 glycoproteins. Surprisingly, CD1 receptors are found to present these non-peptide antigens to a distinct subset of T cell so called as Natural Killer T cells (NKT cells). Furthermore, owing to the broader role played by CD1d in B lymphocytes for instance the requirement of CD1d expression by B cells for the prolonged antibody responses, we focused our studies on this molecule, which is the only CD1 isoform expressed by mouse. Most of the studies are regarding the endocytic trafficking behavior of these receptors but the topography and the landscapes of these receptors on the surface of antigen presenting cells (APC) remain unexplored. Studies carried out by biochemical methods tend to associate CD1d with Major Histocompatibility complex II (MHC II) and β2-microglobulin in the case of B lymphocyte, dendritic cells and epithelial cells but no biophysical techniques have been used till now to define these characteristics. Since, fluorescence resonance energy transfer (FRET), a spectroscopic ruler, can measure intermolecular protein interactions below 10 nm; we assessed these presumed relationships by FRET. The results reveal some exciting harmony between these proteins. We found FRET values of ~19, ~17 and ~12 % between CD1d and MHC I heavy chain, β2-microglobulin and MHC II respectively indicating the facts of association. We also found that majority of these molecules are localized in the nano-domains of lipid rafts. Further experiments are underway to define these characteristics to the biological significance. We believe our findings would provide better insights into the biological phenomenons like endocytosis and lipid antigen presentation by CD1d receptors which could provide essential clues in the treatment of many diseases.

Cardiac electrophysiological effects of rosiglitazone in canine ventricular cells

Ferenc Ruzsnavszky 2nd year PhD student
Department of Physiology
Tutor: János Magyar

The antidiabetic drug rosiglitazone has a widespread clinical application, but there is little information about its possible effects on cardiac cells in larger mammals. Therefore in the present study we examined the concentration-dependent effects of rosiglitazone on action potential morphology and the underlying ion currents. Enzymatically isolated canine ventricular cardiomyocytes were used for conventional whole cell patch clamp, and action potential voltage clamp techniques for our experiments. At concentrations higher than 10 μM rosiglitazone decreased the maximum velocity of depolarization and the amplitude of phase-1 repolarization. Action potential duration was affected little by the drug. However, APD_{50} was significantly shortened by 30 μM, while APD_{90} was lengthened by 100 μM of rosiglitazone. These changes in action potential morphology were accompanied by depression of the plateau potential. These effects of rosiglitazone developed rapidly and were reversible upon washout. Rosiglitazone suppressed several transmembrane ion currents in a concentration-dependent manner under conventional voltage clamp conditions and altered their kinetic properties. The EC_{50} value for this inhibition was 25.2±2.7 μM for I_{to}, 72.3±9.3 μM for I_{Kr}, and 82.5±9.4 μM for I_{Ca}, with Hill coefficients close to unity. I_{K1} was not affected up to concentrations of 100 μM of rosiglitazone. Suppression of I_{Ca}, I_{Kr}, and I_{to} has also been confirmed by action potential voltage clamp measurements. The observed changes in the densities and kinetic properties of ion currents may carry proarrhythmic risk in case of roziglitazone intoxication.

PP2A dephosphorylates ezrin-radixin-moesin (ERM)-binding phosphoprotein 50 at the mitotic phase of the cell cycle

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Ezrin-radixin-moesin (ERM)-binding phosphoprotein 50 (EBP50/NHERF-1) is a membrane-cytoskeleton linking protein that binds to integral membrane proteins, various receptors, channels, and cytoplasmic signal proteins. It has two PDZ (PSD-95/Disk-large/ZO-1 homology) and a C-terminal ERM-binding domain. In polarized epithelial cells it is found in the apical membrane and interacts with the Na^+/H^+-exchanger (NHE3) to confer cAMP-mediated inhibition of Na^+/H^+-exchange. EBP50 is a subject to phosphorylation by several kinases. It is phosphorylated in HeLa cells during mitosis on serines 279 and 301 by cyclin-dependent kinase, Cdk1. The dephosporylation of the EBP50 is still unclear. In our study BPAE (Bovine Pulmonary Artery Endothelial) cells were arrested in G2/M phase by nocodazole treatment. In the cell lysates we detected phosphorylation of EBP50. After releasing the cells from the mitotic block specific phosphatase inhibitors were added to the medium. Okadaic acid (5 nM) was able to maintain the phosphorylated state suggesting that PP2A dephosphorylates EBP50. Furthermore, the A regulatory and the C catalytic subunits of PP2A co-immunprecipitate with EBP50, but no specific interaction was detected between EBP50 and the PP1c isoforms. We found different localization of EBP50 in interphase and in mitotic cells by immunofluorescent staining. EBP50 was amplified from bovine cDNA and cloned into pCMV-myc mammalian expression vector to identify interacting partners.

Effects of nicotinic acid on the biological functions of human sebocytes

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Due to its well known lipid-lowering activity, nicotinic acid (NA) is widely applied in the treatment of different dyslipidemias. It has recently been shown that this beneficial antilipolytic action is mediated by a G protein coupled receptor (HM74A) expressed by adipocytes. Since sebocytes, as well as adipocytes, are characterized by a highly complex lipid homeostasis, in the current study we investigated the potential impact of NA on the biology of human sebaceous gland-derived cells.

First, using human, immortalized SZ95 cells, we showed that sebocytes express HM74A both on mRNA (Q-PCR) and on protein levels (immunocytochemistry, Western blot). Since we have successfully demonstrated the presence of the NA receptor, we continued our experiments by its functional investigation. Using colorimetric MTT assay and fluorescent Nile Red staining we have shown that up to 100 μM neither viability, nor basal neutral lipid (sebum) synthesis was influenced by NA-treatment.

Under certain circumstances the sebum production of the sebocytes can be dramatically increased, and this seborrheic state may be the first step in the pathogenesis of acne vulgaris. To investigate the effects of NA in seborrhea, we co-administered it with various "lipogenic" substances (endo-cannabinoids, arachidonic acid and linoleic acid-testosterone combination), which act through different signaling pathways. As we found that NA was able to normalize the lipid synthesis of the sebocytes in all cases, we can conclude that it may have a universal sebostatic action.

As the increase of the $[Ca^{2+}]_{IC}$ of the sebocytes may result in similar "sebostasis signal", we have investigated the effects of NA on the Ca^{2+}-homeostasis of the SZ95 cells. Using a fluorimetric Ca^{2+}-imaging technique (Fluo4-AM, FlexStation) we have shown that NA transiently increased the $[Ca^{2+}]_{IC}$ of the cells, and the source of the incoming Ca^{2+} was mostly, but not exclusively the extracellular space.

Collectively, our findings suggest that NA could be successfully used in the treatment of such a common skin disorder as acne, which is characterized by pathologically elevated sebum production of the sebaceous glands.

Investigation of the effects of PARP-2 depletion on SIRT1 activation

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SIRT1 is a NAD^+-dependent deacetylase that deacetylates lysine residues of proteins at the expense of a NAD^+ molecule, hence NAD^+ availability modulate SIRT1 activity. SIRT1 activation induces mitochondrial biogenesis. Therefore SIRT1 senses the cellular energy status through sensing cellular NAD^+ and it is capable of counteract the scarcity of energy through impacting on mitochondrial metabolism.

We observed enhanced mitochondrial biogenesis and higher SIRT1 activity in poly(ADP-ribose) polymerase-2 (PARP-2) knockout mice. We have shown that PARP-2 deficiency increases SIRT1 activity in cultured myotubes through modulating SIRT1 expression. Our investigation have shown PARP-2 to act as a repressor of SIRT1 promoter, therefore PARP-2 depletion induced SIRT1 expression.

We further tested our observations in model of Doxorubicin (DOX)-induced cardiovascular damage where resveratrol (a SIRT1 activator) was proved to be protective. DOX enhances free radical production through providing a pathway for the leakage of electrons from mitochondria. Free radicals induce DNA damage, PARP activation, and cell dysfunction, therefore PARP-1 knockout mice were protected against DOX-induced damage.

Aortae from PARP-2 knockout mice were partially protected against DOX-induced damage of the smooth muscle cells. Since PARP-2 possesses PARP activity we hypothesized that the protective phenotype may stem from similar mechanisms as in the PARP-1 knockout mice. However we were unable to detect large scale reduction in PARP activity and NAD^+ depletion upon knockdown of PARP-2.

We examined whether the induction of SIRT1 upon PARP-2 depletion may be responsible for the protective phenotype. Indeed in aortae and in cultured smooth muscle cells (MOVAS cells) depleted of PARP-2 we observed the induction of mitochondrial DNA and the expression of SIRT-1 target genes. In both models SIRT1 expression was induced. We have shown that upon PARP-2 depletion the activity of the SIRT1 promoter increases. In summary, SIRT1 activation enhanced mitochondrial biogenesis that through stabilizing mitochondrial activity provided protection against DOX-induced mitochondrial damage.

Characterisation of the chondrifying high density cultures of the BMP2-producing C3H10T mouse embryonic mesenchymal cell line

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Based on previous results of our laboratory, we identified a type of the ionotropic glutamate receptors, which has mainly been described on excitable cells formerly, the NMDA receptor, as an important factor of chondrogenic differentiation in embryonic chicken cells.
At the beginning of our current experiments, we optimalised the culturing circumstances of the BMP2-expressing C3H10T cells for their chondrogenic differentiation, then our next goal was to observe the expression pattern of the different NMDA receptor subunits in the differentiating cultures.
In our model system, chondrification of the high density (HD) cultures spontaneously occurs in 15 days. We carried out our gene expression studies by the means of RT-PCR reactions, using primers designed based on the sequences that are available in the NCBI database.
We were able to detect metachromatic cartilage matrix from the tenth day of culturing with the acidic dimethyl-methylene blue staining of cultures harvested on different days of culturing. At the same time, we confirmed cartilage formation with RT-PCR experiments monitoring the expression of collagen type II, aggrecan and Sox9. With the same method, we also detected NR2B, NR3A and NR3B subunits. However, in the case of NR1, which is dogmatically essential for the formation of functional NMDA receptors, we could detect very low levels of expression. Experiments studying protein expression of the channel subunits and the evaluation of the effect of transient gene silencing of the NR1 subunit with siRNA transfection on cartilage formation are currently in progress.
This murine chondrogenesis model, while it carries the advantages of a mammalian system, is an adequate alternative for the chicken primary chondrifying cultures that have been applied for a long time in our laboratory. The fact that we were able to detect NMDA receptor subunit expression in both systems underlines the chondrogenic role of this type of ion channels.
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The possible regulatory role of TRPC6 channel in mouse and human immortalized podocytes

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A leading cause of chronic kidney disease in children and adults is focal and segmental glomerulosclerosis (FSGS), the developement of which is associated with a „gain-of-function" mutation or acquired hyperfunctioning of transient receptor potential canonical-6 (TRPC6) channel; based on the data found in the literature. TRPC6 is a non-selective, Ca^{2+}-permeable ion channel which can be activated by diacylglycerol (DAG) in a membrane-delimited fashion. Several mediators can modulate the activation of this channel, but the details of the regulation and the mechanism of the development of podocyte damage is still unknown. Therefore in our current study we wanted to analyse the expression and functional properties of TRPC6 in mouse and human podocytes.

Our experiments were performed on mouse and human podocytes. We showed the presence of functionally active TRPC6 channels since the application of DAG analogs (1-oleoyl-2-acetyl-sn-glycerol; 1,2-dioctanoyl-sn-glycerol) increased the $[Ca^{2+}]_{IC}$ in a dose-dependent manner (Fluo4-AM, FLIPR). In the next step we investigated whether this activation can be modulated by different agents. We used 30-minute pretreatment with different inflammatory agents (histamine, arachidonic acid, prostaglandine E2) and other mediators (ATP, bradykinin), the general PKC activator PMA and insulin. Our functional studies revealed that PMA and bradykinin inhibited the increase of $[Ca^{2+}]_{IC}$, however the inflammatory agents did not modify the effect of DAG analogs. Moreover, long term (24 hours) administration of insulin and arachidonic acid significantly increased the mRNA expression of TRPC6, while the other agents did not affect its expression. These results suggest that the PKC system may play an important role in the regulation of TRPC6 activation. Therefore in the next phase of our experiments we plan to systematically investigate the effect of PKC isoforms on the functionality of TRPC6.

Our results suggest that the direct modulation of TRPC6 by PKC isoforms may play an important role in the pathogenesis of glomerulopathies, which are assosiated with acquired podocytopathy and proteinuria.

MHC I organizes protein clusters and inhibits IL-2/IL-15 signaling in human T cells

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Major histocompatibility class I and II glycoproteins and interleukin-2 and -15 receptors form supramolecular clusters in lipid rafts of FT7.10 T lymphoma cells. IL-2 and IL-15 cytokines play an important role in T cell activation and immunological memory, whereas MHCs are known for their role in antigen presentation.
We applied RNAi to silence the expression of MHC I in order to study its possible role in receptor assembly and function. FRET data indicated that the association of IL-2R and IL-15R with MHC I as well as between IL-2R and IL-15R weakened. FCS indicated an increase of receptor mobility also suggesting the partial disassembly of the clusters. MHC I gene silencing lead to a remarkable increase of IL-2/IL-15 induced phosphorylation of STAT5 transcription factors, hinting at inhibition of IL-2/15 signaling by MHC I. In search for the molecular background of this inhibition we checked IL-2 binding and the assembly of the receptor complex. IL-2 binding is increased in MHC I knock-down samples, which partially explains the detected increase of STAT5 phosphorylation. The association of IL-2Ralpha with IL-2R beta did not show a difference as compared to the control.
Our results suggest that MHC I plays an organizing role in maintaining supramolecular receptor clusters and inhibits IL-2R signaling, revealing a nonclassic new function of MHC I beyond its classical role in antigen presentation.

Multiplexed Glycoanalytics: bioinformatics assisted rapid and confident N-Glycan structural elucidation

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Structural analysis of glycoprotein-derived carbohydrates is a challenging task, mainly due to the extremely high diversity of glycans that arises from the complexity of the genetic and environmental factor sensitive glycan-processing pathways. New high-resolution separation methods are crucial to provide alternatives for comprehensive analysis of complex glycan pools containing positional and/or linkage isomers. Capillary electrophoresis (CE) is a high performance separation technique that holds the promise to fulfill this quest. Albeit, CE offers high separation efficiency and resolving power, as well as very short separation times and the option of easy multiplexing, a comprehensive structure-mobility database is greatly needed to readily elucidate glycan structures directly from electrophoretic mobility data. Both experimental and computational model-based approaches were applied to build up such a CE glycan database that eases structural elucidation and also enables the identification of unknown carbohydrate species based on their sole electrophoretic mobility data.
N-glycans attached to the CH2 domains of the Fc or antigen binding regions of IgG play an important role in stabilising and modulating the antibodies activity. Exhaustive elucidation of 32 IgG N-glycans using a combination of weak anion exchange enrichment and exoglycosidase array digestion with subsequent profiling exceeded 48 hours. Pursuing increased throughput and associated structural annotation confidence, we compared the 1.7 μm hydrophilic interaction chromatography (HILIC) phase for UPLC with CE-LIF for the rapid and comprehensive characterisation of N-glycans released from healthy human serum polyclonal IgG. Combination of the data individually generated using each technique demonstrated that complete structural annotation was possible in less than 20 minutes due to the advantageous orthogonality of the separation mechanisms. The parallel use of both analytical techniques offers a powerful platform for rapid and comprehensive characterization not only for IgG N-glycosylation but also other glycoproteins of therapeutic importance.

Fractionation of the human plasma proteome for monoclonal antibody proteomics based biomarker discovery

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Monoclonal antibody proteomics is a novel methodology to discover proteins with biomarker potential, but requires subsequent antigen identification steps. The identification of medium or lower abundant proteins needs a large amount of sample to provide proper amount of antigens for the identification process. The aim of the project was to generate a comprehensive analyte library representing the human plasma proteome, addressing the presently existing 11-12 order of magnitude differences, which make isolation and identification of low concentration proteins extremely difficult. The fractions of the analyte library can then be readily used to accommodate the protein identification process for monoclonal antibody proteomics based biomarker discovery. First the large human serum albumin and immunoglobulin content were removed from 500 mL normal pooled human plasma by specific chromatography based partitioning methods and the resulting depleted plasma was pre-fractionated by ammonium sulfate precipitation. Each fraction was then further separated by size exclusion chromatography, followed by cation and anion exchange chromatography steps. The 20 most concentrated ion exchange chromatography fractions were further separated by hydrophobic interaction chromatography. This process resulted in 783 fractions with the average protein concentration of 1 mg/mL. All chromatography and precipitation steps were carefully designed with the purpose of maintaining the native forms of the intact proteins throughout the fractionation process. The separation route of vitamin D-binding protein (an antibody proteomics lead antigen) was followed in all major fractionation levels by dot blot assay in order to identify the library fraction it accumulated in and the identity of the antigen was verified by Western blot.

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Nuclear localization, function and regulation of myosin phosphatase

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The myosin phosphatase (PP1M) holoenzyme is a member of the family of serine/threonine specific protein phosphatases. It composed of a 38 kDa type 1 protein phosphatase catalytic subunit (PP1c), a 130/133 kDa MYPT (myosin phosphatase targeting subunit) and 20 kDa subunits. Myosin is a classical substrate of PP1M but several non-muscle substrates have also been identified. MYPT was found to be localized not only in the cytosol and cytoskeleton but in the nucleus in rat aortic smooth muscle cells, primary cultures of neuronal cells as well as in human hepatocarcinoma (HepG2) cells. Our goal is to investigate the nuclear functions of PP1M by determining the subnuclear localization and interaction proteins of MYPT. Subnuclear fractions of HepG2 cells were analysed by Western blotting and protein phoshatase enzyme activity assays. MYPT1 showed localization in spliceosomes (nuclear compartments of cell) by confocal microscopy suggesting that PP1M may play a role in mRNA splicing. Flag-MYPT pull down assays were carried out using nuclear fractions of HepG2 cells. The eluates were applied for silver staining followed by mass spectrometry. Numerous potential nuclear MYPT1-interacting proteins were identified such as smoothelin-like protein 1 (SMTNL1), which is known to inhibit the activity of PP1M by binding to MYPT1. To determine the region of MYPT1 is responsible for the protein-protein interaction with SMTNL1 we carried out surface plasmon resonance-based technology using purified Flag-tagged-SMTNL1 and His-tagged truncated mutants of MYPT1. The data prove that the major SMTNL1-binding site on MYPT1 is the N-terminal ankyrin repeate. We would like to confirm these results with in vitro immunoprecipitations, too. We also plan is to investigate the effect of phosphorylation of SMTNL1 or MYPT1 on their protein-protein interaction, and to study the relationship between MYPT and its novel nuclear partners.

Effects of nifedipine treatment on calcium homeostasis of urinary bladder smooth muscle cells

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Calcium channel blockers are frequently used to treat hypertension, ischaemic heart disease, peripheral atherosclerosis and other vascular disturbances. Previous studies showed acute and chronic effects of the L-type calcium channel blocker on heart muscle and vascular smooth muscle, but we have limited information about urinary bladder smooth muscle. Our aim is to study the acute and chronic effects of the calcium channel antagonist nifedipine on the calcium homeostasis of urinary bladder smooth muscle cells.

Our measurements were performed on smooth muscle cells from canine urinary bladder. For studying the immediate effects of nifedipine, we prepared primary urinary bladder smooth muscle cell cultures from control dogs. The chronic effects were examined on cultured urinary bladder smooth muscle cells from the animals were treated daily with one tablet of „Adalat GITS 60 retard" for 4 weeks, then cultured their urinary bladder smooth muscle cells. We measured the intracellular calcium concentration of the cultured cells at 37 °C using the fluorescent dye FURA2- AM. We detected ATP evoked intracellular calcium transients in smooth muscle cells from both control and nifedipine treated dogs.

Investigation of the acute effects of nifedipine included repetitive ATP applications to the control smooth muscle cells. We applied 180 μM ATP alone, then in the presence of 10 μM nifedipine. The nifedipine decreased the amplitude of the ATP evoked calcium transients, indicating the contribution of the L-type calcium channels to the ATP evoked intracellular calcium increase.

Examination of RasGRP3 expression on human breast cancers and breast-derived ductal adenocarcinoma cell lines

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RasGRP3 is a member of the Ras guanine nucleotide releasing protein (RasGRP) family of the Ras-specific guanine nucleotide exchange factors. These proteins play an important role in the regulation of the activity of Ras signaling pathway, which is known to be constitutively active in many cancer types. The RasGRP3 proteins have a potential oncogenic effect, since recent investigations have highlighted the amplification of the protein's gene in many malignant cancer types, for example in Burkitt's lymphoma, pre-B-cell leukemia and prostate adenocarcinoma.

In the light of this potential oncogenic effect our goal was to examine the possible change in the expression of RasGRP3 and its phosphorylated form in one of the most malignant cancer types, the breast-derived ductal adenocarcinoma.

In our examination using immunohistochemistry we successfully proved that the expression RasGRP3 and phosphoRasGRP3 are increased in direct proportion to the cancer grade on the human ductal adenocarcinoma samples. We found that RasGRP3 is expressed typically in the cell's cytoplasm, while the phosphoRasGRP3 staining shows strong nuclear reaction. Using Q-PCR and Western Blot we demonstrated the expression of the RasGRP3 and its phosphorylated form in mRNA and protein levels on different primary (BT-474) and metastatic (MCF7, T-47D, SK-BR-3, MDA-MB-453, JIMT-1) breast-derived cell lines. We found that the RasGRP3 expression in the BT-474 cell line is lower then that found in the metastatic cell lines. These results suggest the RasGRP3 may has importance in the development of metastases.

In our further studies we would like to create RasGRP3 knockdown cultures on the cell lines mentioned above, because we are curious about what kind of role this protein plays in the cell's viability, proliferation, apoptosis, tumorigenicity and in the development of resistance against different chemotherapeutic drugs.

Investigation of the low count detectability in nuclear medicine images using human and model observers

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Aims: Studies in nuclear medicine frequently require the measurement of very low activity lesions in a background region. The detectability depends on the number of property of the lesion (size, shape, statistic …), the background (quantum noise from the imaging process and anatomical variability) and the observer performance. The aim of this work was to define an appropriate FOM parameter to assess the performance of the low count detectability at different imaging situations. We also investigated the differences between the human and mathematical model observers.
Methods: We used simulated images in this work. The images were generated with Poisson statistics, including a set of predefined parameters, as background activity level, signal (lesion) size, signal amplitude (contrast). 10000 simulated images were investigated by human observers (HuO) using an own software tool, testing whether these signals (lesions) are visible or not. For model observers the images were compared on the basis of the channelized Hotelling observer (CHO) using predefined channels. The results and the efficiency of human and model observers were compared using the receiver operating characteristic (ROC) analysis that matches true positive versus false positive rates.
Results: For the HuO cases we calculated the contrast, the SNR and the lesion area corrected SNR ROC curves. The related AUC (area under the curve) values were 0.722, 0.781 and 0.928, respectively. If we used the CHO model the AUC value of the ROC curve was 0.938. We also applied the CHO to investigate the detectability on the lesion size. We generated the related ROC curves for the following lesion area intervals: 0-4, 4-6, 7-11 and 11-15 pixels. The resulted AUC values were 0.631, 0.841, 0941 and 0.977, respectively.
Conclusion: The CHO with convenient model parameters can be used as an appropriate FOM to analyze the low count detectability in nuclear medicine images.

CD44-ICAM-1 crosstalk alters MHCI rafts

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Motivation is whether there exist differences in the pattern-forming capabilities of two adhesion molecules of different roles: CD44, mediating „dynamic" adhesion in cell rolling and ICAM-1, mediating „static" adhesion during the formation of immune-synapse. Homo- and hetero-associations of CD44, ICAM-1 and the MHCI is investigated on the nm- and μm- distance levels on LS174T colon carcinoma cells in two different conditions of lymphocyte homing: (1) With IFNγ and TNFα, both lymphokines up-regulating the expression level of MHCI and ICAM-1 and down-regulating that of CD44. (2) Crosslinking of CD44 and ICAM-1 representing receptor engagement. The observations are explained by assuming the existence of a kinase cascade-level crosstalk between the CD44 and ICAM-1 molecules which manifests in characteristic complementary changes in the properties of cell surface receptor patterns. For the characterisation of cluster morphology new colocalization approaches were developed: (i) „number of first neighbours" distribution curves, (ii) „acceptor photobleaching FRET-fluorescence intensity fluctuation product" correlation diagrams.

Effects of selenium on the contractile properties of mouse skeletal muscles

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It was shown previously that selenium compounds play a significant role in many physiological functions of the organs, although there is a narrow border between the effective and toxic dose. It has an important anti-oxidant effect, plays role in certain cardiovascular disease prevention, and has positive impact on the cognitive properties. However the effect of selenium in skeletal muscle have not been studied yet.

In our study we examined the effects of different selenium compounds on muscle properties of mice fed with selenate and NanoSel compounds in different concentrations for two weeks. NanoSel produced At the University of Debrecen, Centre of Agricultural Sciences, NanoFood Labor of Bio- and Environmental-genetics Department, which contains 100-500 nm-sized elemental selenium in organic selenium pellets. Selenate bounds to selenoproteins, while NanoSel transformed into selenit in vivo, so it's probably less toxic than selenate. The selenium compounds were used in the concentration of 0.5; 5 and 50 ppm.

During our measurements, we measured force in soleus and extensor digitorum longus (EDL) muscles of the mouse and we detected changes in intracellular Ca^{2+} concentration on single fibers from flexor digitorum brevis (FDB) muscle loaded with fluorescence dye.

Both forms of selenium in both muscle types significantly increased the amplitude of single twitches in a concentration dependent manner (for example in EDL muscle from 99.5±4.7 mN to 127.8±5.1, 140.7±3.6 and 190.8±10.6 mN in case of 0.5, 5 and 50 ppm selenate, respectively). The fatigue of both muscles was reduced by both selenium compounds in the highest concentration during tetanus series (EDL 31±11, 49±5 and 46±1%; soleus 39±11, 63±5 and 51±6% control, selenate and NanoSel, respectively). The resting intracellular calcium concentration of the FDB fibers was identical in all group measured. In contrast the amplitude of the calcium transients evoked by KCl depolarization increased significantly from the control 216±22 nM to 326±60 nM in the presence of 0.5 ppm NanoSel. Our results suggest that selenate and NanoSel improve the contractile properties of skeletal muscles.

Binding of trastuzumab to ErbB2 is inhibited by a high local density of hyaluronan

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Background: Overexpression of ErbB2 in breast tumors is associated with poor prognosis and is a target of receptor-oriented cancer therapy. Trastuzumab (Herceptin) is an anti-ErbB2 antibody used in the treatment of ErbB2-overexpressing breast cancer, but the development of resistance is currently inevitable. We have previously shown in cell cultures and mouse xenograft experiments that masking of ErbB2 by the cell surface mucin MUC-4 or by hyaluronan leads to diminished binding of trastuzumab and consequent trastuzumab resistance. However, such correlations have not been established for human tumor samples. In the current work we investigated ErbB2-overexpressing breast cancer tissue samples and correlated the binding of trastuzumab to ErbB2 with the local density of hyaluronan.
Methods: ErbB2 in frozen tissue sections were dual stained with a fluorescent antibody against the intracellular domain (OP15) and with trastuzumab. Hyaluronan was visualized by labeling with HABC (hyaluronic acid binding complex). Immunofluorescence images were taken by confocal microscopy.
Results: We calculated the relative binding of trastuzumab by normalizing the fluorescence of trastuzumab with that of OP15. We found that the relative binding of trastuzumab showed a negative correlation with the local density of hyaluronan. Analysis of the relationship between clinical trastuzumab resistance, patient survival and hyaluronan-mediated masking of ErbB2 is in progress.
Conclusions: Although hyaluronan is by no means the only molecule contributing to trastuzumab resistance and ErbB2 masking, our results confirming its role in inhibiting trastuzumab binding in human tissue samples has both diagnostic and therapeutic implications.

Characterization of R5 glycogen binding subunit homolog in Drosophila

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In the Drosophila genome database we identified a gene (CG9238) encoding a mammalian R5 glycogen binding subunit homologue protein (R5h). R5 is a regulator subunit of protein phosphatase 1 (PP1) catalytic subunit, which has an important role in mammalian glycogen metabolism. By the computational analysis of the primary structure of Drosophila R5h protein, we could detect a conservative PP1 binding motif and glycogen binding domain.
In order to control the structural theory, we used yeast two-hybrid system to confirm R5h can form protein-protein interaction with PP1. The result of the experiment showed that all Drosophila isoform of PP1 catalytic subunit are able to interact with R5h protein.
We expressed and purified R5h in Escherichia coli and demonstrated that recombinant protein can bind to glycogen.
On the basis of the CG9238 gene structure probably two mRNAs can transcribed. In Drosophila embryos, we can detect just the shorter mRNA with RT-PCR assay. During Drosophila ontogeny, this mRNA is detectable in embryos, larvae and adult flies.
To determine the function of R5h, we applied an insertion mutant named EY10816, which carries a P-element in the gene 5' non-coding region. The presence of the P-element does not influence the transcription of the gene, thus we mobilized the P-element with transposase enzyme. Random excisions occur during the removal of the P-element. In one case (EY 10816/81), notable deletion was created in the CG9238 gene. We defined the size of the deletion with PCR (2kb) and exact position with DNA sequencing. We found that the deletion eliminate the 5' region of the gene, and exon 1 and 2 as well. We verified the absence of shorter mRNA in the EY10816/81 homozygous deletion mutant with RT-PCR method. The deletion mutant is viable, but based on our preliminary investigations it seems there are differences between wild type and mutant reserved nutrient distribution in Drosophila oocytes. In order to verify our results we are planning to rescue the mutant fenotype and developing a specific antibody to characterize the role of R5h in Drosophila oogenesis.

HaCaT keratinocytes exhibit spontaneous intracellular calcium transients

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Tutor: László Csernoch

In our experiments we examined the $[Ca^{2+}]_i$ in HaCaT keratinocytes in scratch assay. Our aim was to describe the alterations of the Ca^{2+} homeostasis in two different areas: close to the scratch and far from it. The keratinocytes were cultured on glass coverslips. The scratch was made with a 10 µl pipette tip. We monitored the effect of two phosphatase inhibitors (iso-okadaic acid and calyculin A) on the resting intracellular calcium level and we tested calcium channel blockers (nickel- (5 mM) cadmium- (5 mM) and lanthanum-ions (250 µM)) and calcium free extracellular solution also.
We observed spontaneous calcium transients with confocal microscopy using fluo-4-AM loaded cells. The pictures were analysed with image J program and a special program developed in our laboratory. In addition, the resting $[Ca^{2+}]_i$ was measured on PTI Delta Scan system. In that case the cells were loaded with ratiometric Fura-2-AM dye.
We observed significant difference in the resting $[Ca^{2+}]_i$ between the two areas and this difference was still observable after having treated the cells with the phosphatase inhibitors. The phoshatase inhibitors changed the resting calcium concentration significantly in the cells far from the scratch: calyculin A decreased the resting $[Ca^{2+}]_i$, while iso-okadaic acid increased it. In presense of calcium channel blockers nickel- and lanthanum-ions the spontaneous calcium transients did not disappear. In case of the cadmium-ion the cells were shrank and the spontaneous calcium transients disappeared.

Trisk 32 regulates IP3 receptors in rat skeletal myoblasts

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To date four isoforms of triadins have been identified in rat skeletal muscle. While the function of the 95 kDa isoform in excitation-contraction coupling has been studied in detail, the role of the 32 kDa isoform (Trisk 32) remains elusive.

Here Trisk 32 overexpression was carried out by stable transfection in L6.G8 myoblasts. Co-localization of Trisk 32 and IP_3 receptors (IP_3R) was demonstrated by immunocytochemistry and their association was shown by co-immunoprecipitation. Functional effects of Trisk 32 on IP_3-mediated Ca^{2+} release were assessed by measuring changes in $[Ca^{2+}]_i$ following the stimulation by bradykinin or vasopressin. The amplitude of the Ca^{2+} transients evoked by 20 mM bradykinin was significantly higher in Trisk 32-overexpressing ($p<0.01$; 426±84 nM, n=27) as compared to control cells (76±12 nM, n=23). The difference remained significant ($p<0.02$; 217±41 nM, n=21 and 97±29 nM, n=31, respectively) in the absence of extracellular Ca^{2+}. Similar observations were made when 0.1 μM vasopressin was used to initiate Ca^{2+} release. Possible involvement of the ryanodine receptors (RyR) in these processes was excluded, after functional and biochemical experiments. Furthermore, Trisk 32 overexpression had no effect on store-operated Ca^{2+}-entry, despite a decrease in the expression of STIM1. These results suggest that neither the increased activity of RyR, nor the amplification of SOCE are responsible for the differences observed in bradykinin- or vasopressin-evoked Ca^{2+} transients, rather, they were due to the enhanced activity of IP3R. When IP_3R was activated directly by 50 μM thimerosal, the amplitude and maximal rate of rise of the transients were similar in control and Trisk 32-overexpressing cells, which suggests that the conductance of the receptors did not change.

Thus Trisk 32 not only co-localizes with, but directly contributes to the regulation of Ca^{2+} release via IP_3R, possibly by increasing the IP_3-sensitivity of the receptor.

In vivo test of a highly stable antifungal protein (PAF) against lung aspergillosis

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The Aspergillus fungi can cause a severe lung disease (aspergillosis) in people with an attenuated immune system, so it would be important to find new drugs against this species.
Our research topic was to investigate the effects of the antifungal peptide produced by the Penicillium chrysogenum (PAF) in animal experiments.
In our experiments adult mice were inoculated intranasally with PAF. Animals were randomly divided to six groups in which they got PAF in 0, 2, 10, 50, 100 and 1091 μg/ml concentration once a week for 5-8 weeks.
Animals did not die during the experiment and no side effects were observed. We did not find any pathological reaction during the histological experiments.
In the next experiment the effect of the drug was examined on the skin in an irritative dermatitis model. PAF was spread on the ears of the mice in an ointment. There were 3 groups – negative control, PAF and positive control (phorbol-12-myristate-13-acetate – PMA).
The PMA caused a significant expansion of the ears (57.5±29.2 μm) compared to the control (22.5±5.0 μm) while PAF did not (23.8±9.2 μm). Histological reaction was present only in the case of the positive control.
We examined the effects of PAF with a small animal PET camera. Animals were treated with PAF, LPS (lipopolysaccharide) or physiological saline intranasally. The FDG uptake was significantly higher in the LPS group compared to the control and the PAF group. So PAF did not cause inflammation in the lungs of the mice.
The next experiment was the infection of the immune suppressed mice with Aspergillus spores. The spores were inoculated intranasally. This model will be used to examine the curative effect of PAF in lung aspergillosis.
Consequently we did not find any toxic effect of PAF either in systemic or in local external treatment. In our next experiment the effect of PAF will be investigated on the procession of the disease in infected animals by Aspergillus.

Electrophysiology of differentiating chondrocytes

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Chondrocytes are non-excitable cells and little is known about their plasma membrane ion channels. We aimed to identify these channels and establish their roles in the process of chondrogenesis.
Our model system is a high density chondroprogenitor cell culture isolated from limb buds of chicken embryos. Using whole-cell patch-clamp we detected inward and outward ionic currents whose amplitude and characteristics depended on the time elapsed since isolation. Using ion substitution experiments we identified the channels responsible for the currents as Na^+ and K^+ channels, respectively.
The voltage-dependence of steady-state activation and inactivation of the Na^+ channels were characterized by $V_{1/2,a}$ = –36.8 mV and $V_{1/2,i}$ = –72.4 mV. These channels were reversibly blocked by tetrodotoxin (K_d = 12 nM). These values suggested the presence of NaV1.4 or NaV1.3 channels. mRNA and Western-blot experiments proved the expression of NaV1.4, but not of NaV1.3 channels.
Outward K^+ currents showed high heterogeneity in amplitude, inactivation kinetics and TEA sensitivity suggesting the presence of different K^+ channels. Based on prior electrophysiological characterization we presumed the presence of Kv1.1, Kv4.1 and voltage independent 2-pore channels contributing to the K^+ conductance. mRNA and Western blot analysis confirmed our hypothesis. These results were further supported by computer simulations, in which measured K^+ currents and their TEA sensitivity were modeled as the sum of the currents through the three different channel types in various ratios.
The membrane potential distribution of the cells was highly K^+-dependent and showed bimodal distribution: in one population, the mean membrane potential was more positive (–39.0 ± 4.1 mV) while the other population was much more hyperpolarized (–72.2 ± 1.5 mV). In several cells a transition to the hyperpolarized state could be observed during recording suggesting the activation of the large, highly K^+-selective, 2-pore- conductance.
Our further aim is to characterize this voltage independent component of the K^+ current and to study its contribution to the differentiation process.

Biophysical investigation of IL-9R assembly and function in human T-lymphoma cells

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Interleukin-9 is a multifunctional cytokine with pleiotropic effects on T cells. Numerous observations suggest its potential role in T cell oncogenesis, but the exact function of IL-9 in T cell responses is still unclear.
The IL-9R consists of a ligand-specific α-subunit and the common γ_c-chain shared with other cytokines, including IL-2 and IL-15, important regulators of T cells. Our group demonstrated previously the preassembly of the heterotrimeric IL-2R and IL-15R complexes and their participation in common supramolecular clusters with MHC molecules in lipid rafts of human T lymphoma cells.
We can hypothesize that other members of the γ_c cytokine receptor family, such as the IL-9R complex, may also fulfill their tasks in a similar environment, maybe in the same superclusters. In order to test this hypothesis we performed FRET and CLSM experiments to investigate cell surface organization and association patterns of IL-9R in different human T lymphoma cell lines. By using these approaches, we demonstrated co-expression and molecular scale association of IL-9Rα with IL-2R and MHC molecules in common membrane domains, presumably in lipid rafts. Molecular scale interactions of IL-9Rα with γ_c and IL-2Rα were modulated upon IL-9 binding, implying a conformational change within the receptor complex. In addition, cholesterol depletion by methyl-β-cyclodextrin affected the efficiency of IL-9R-mediated signaling as revealed by flow cytometric detection of STAT1 phosphorylation.
Our results along with our previous data for the IL-2/15 receptor system hint at the possibility that preassembly of the receptor complexes in common membrane microdomains with MHC glycoproteins may be a general property of γ_c cytokines in T cells, and this kind of organization may promote optimal signaling and subunit switching among them.

Application of SPR technique to characterize the interaction of membrane-permeable enzyme inhibitors with lipid micelles

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To develop their intracellular influence, the membrane-permeable inhibitors have to diffuse through the membrane. The attachment of the inhibitors to the membrane lipids can be a primary step in their transport process. The interaction between the inhibitors and lipid micelles can be characterized with surface plasmon resonance (SPR) based detection. Micelles were prepared from lyophilized total lipid extract of brain, heart or liver. Liposomes were captured on L1 Sensor Chip in isolated flow cells. The reference (lipid free) and the three lipid surfaces were blocked with BSA.
We studied the binding of protein phosphatase inhibitors, okadaic acid (OA), microcystin-LR (MC), cyclosporin-A (CsA), tautomycin (TM), cantharidin and epigallocatechin-3-gallate (EGCG), as well as the endothelial nitric-oxide synthase inhibitor nitro-L-arginine methyl-ester (L-NAME) to the lipid surfaces. Cantharidin bound similarly to the three lipid surfaces with respects to both association and dissociation characteristics. In contrast, OA, MC, CsA and EGCG bound with higher affinity to the liver and heart lipids than to the brain counterparts, while the interaction of TM with heart lipids was much weaker compared to the brain and liver surfaces. Plotting the observed resonance unit maximums against the concentration of the inhibitors indicated that the association of EGCG to the lipid surfaces was highly concentration dependent (1-100 μM), which was less characteristic for MC (1-10 μM). The attachment of L-NAME to the lipid surfaces was characterized by similar sensorgrams, and the calculated equilibrium constants for the dissociation are also close. The association of L-NAME to the different lipid micelles was concentration dependent in the total investigated range.
In conclusion, SPR method provides a feasible model to characterize the association of membrane-permeable enzyme inhibitors with lipid surfaces. This method may also be used to describe the „lipid solubility" properties of pharmacological drugs.

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