# 14th Molecular, Cell and Immune Biology Winter Symposium

*organized by the*
*Molecular, Cell and Immune Biology Doctoral School,*
*University of Debrecen*

Debrecen, 7-8 January, 2021
Online Conference

# 14th Molecular, Cell and Immune Biology Winter Symposium

## Programme:

### January 7

**9:00-10:40 Section 1 Chair: András Szabó**
Link: https://unideb.webex.com/unideb/j.php?MTID=m486346bb870f30996cd037a6288d2cfa

***Opening***

9:00-9:10 József Tőzsér

***Plenary lecture***

9:10-9:40 Gábor Szabó:
Communication between the hierarchical levels of chromatin structure

***Regular talks***

9:40-9:55 Sham Jdeed:
The linkage of genomic stability to breast cancer chemoprevention by rexinoid-based drug combinations

9:55-10:10 Csaba Fillér:
The role of histone modifying proteins in double-strand DNA breaks in meiotic Saccharomyces cerevisiae cells

10:10-10:25 Beáta Boros-Oláh:
Exploring new R-loop regulators by barcode RNA-DNA hybrid immunoprecipitation sequencing (BC-DRIP-seq)

10:25-10:40 Éva Márton:
Estrogen mediated expression of miR200 family members in ovarian cells

**10:40-11:00 Break**

**11:00-11:55 Section 2 Chair: Zsolt Sarang**
Link: https://unideb.webex.com/unideb/j.php?MTID=m9d3f43f5de70d9be51be5ad2474dc362

***Introductory lecture***

11:00-11:20 Endre Kristóf:
Batokines – impact on adipocyte browning and metabolic homeostasis

***Regular talks***

11:20-11:35 Rini Arianti:
Thiamine enhances thermogenic activation in human neck adipocytes

11:35-11:40 Abhirup Shaw:
Irisin treatment stimulated the release of CXCL1 but prevented the upregulation of thermogenesis in human primary cervical subcutaneous and deep neck adipocytes

11:40-11:55 Attila Vámos:
Systematic analysis of beige to white transition of human primary subcutaneous adipocytes

**11:55-13:00 Lunch**

**13:00-14:25 Section 3 Chair: Endre Krisóf**
https://unideb.webex.com/unideb/j.php?MTID=mc8bd460ef010417079827d820eaec380

***Introductory lecture***

13:00-13:25 Zsolt Czimmerer:
Prior IL-4 exposure induces chromatin remodeling and the rearrangement of LPS-activated p65 cistrome, leading to synergistic transcriptional activation of a specific gene set in macrophages

***Regular talks***

13:25-13:40 Dóra Bencze:
Investigating the interaction of NLRP3 and Type I interferon pathways in human plasmacytoid dendritic cells

13:40-13:55 Beatrix Ágics:
Modulation of human dendritic cell mediated inflammatory processes by phytochemicals

13:55-14:10 Kolostyák Zsuzsanna:
Egr2 transcription factor is a potential regulator of differentiation and pathogen elimination in alveolar macrophages

14:10-14:25 Domokos Apolka:
Investigation of complex interactions between alternative macrophage polarization signals and HIF-1α signaling pathway

14:25-14:40 Ahmad Alatshan:
All-trans retinoic acid enhances both the signaling for priming and the glycolysis for activation of NLRP3 inflammasome in human macrophage

**14:40-15:00 Break**

**15.00-16:30 Section 4 Chair: Melinda Szilágyi-Bónizs**
Link: https://unideb.webex.com/unideb/j.php?MTID=m9db8a916a89f1a6f75751276758b189a

15:00-15:15 Attila Pap:
Fat transplantation restores metabolism of fatty liver in PPARγ deficient mouse model

15:15-15:30 Kinga Lénárt:
Tissue transglutaminase knock-out preadipocytes and beige cells of epididymal fat origin possess lower mitochondrial functions

15:30-15:45 László Madar:
Establishing of the mutational spectrum of Hungarian patients with familial hypercholesterolemia

15:45-16:00 Rita Szőke-Kovács:
The role of lipid presentation by CD1c in cancer

16:00-16:15 Arpan Chowdhury:
Activation of Nrf2/HO-1/ferritin system attenuates high phosphate-induced calcification of valve interstitial cells

16:15-16:30 László Sós:
Clearance of apoptotic cells in obesity

## January 8

**8:30-10:00 Section 5 Chair: János Mótyán**
Link: https://unideb.webex.com/unideb/j.php?MTID=m047c2f68fc6f41aaeb5be6982b8b5e0c

***Plenary lecture***

8:30-8:55 Endre Barta
Genomics meets Genetics: A combined RNA-seq and WGS analysis at a 10-member rabbit family proves that the expression level at many genes shows intermediate inheritance and these expression differences might be caused by transcription factor binding site variations

***Introductory lecture:***

8:55-9:15 Gergely Nagy:
The collaboration of transcription factors that determine macrophages

***Regular talks***

9:15-9:30 Orsolya Pálné-Szén:
ChIP-seq based determination and comparison of the human and mouse consensus transcription factor binding site sets

9:30-9:45 Ajneesh Kumar:
Examination of antimicrobial and immunomodulatory peptides in Alzheimer's disease using network analysis of proteomics datasets and the DEAMP Database

9:45-10:00 Gyula Hoffka:
Multiscale simulations on the role of conformational heterogenety

**10:00-11:00 Break and Online Poster Viewing Session**
Link: https://unideb.webex.com/unideb/j.php?MTID=m9e75a2a3e59e12cb0295bc2dc2e5e6a0

Rini Arianti: Thiamine Enhances Thermogenic Activation in Simpson-Golabi-Behmel Syndrome (SGBS) Adipocytes

Ahmed Ashour: Study of biotinylated Zbtb46 transcription factor in mesodermal and myeloid cell differentiation using doxycycline-inducible murine pluripotent stem cells.

Boglárka Bodrogi: Differential expression of Nod-like receptors (NLRs) in the skeletal muscle of myostatin mutant compact and wild-type congenic mouse

Péter Dávid: Investigation the influence of agronomic management on the vineyard soil and trunk-bark microbiome of Vitis vinifera from Tokaj-Hegyalja and Pallag-Botanical and exhibitional garden

Péter Fauszt: Phytonutrient-supplementation improved the health of the intestinal microbiota of Cyprinus carpio

Dóra Géczi: Circulating miRNA profiling in plasma samples of glioblastoma patients

Vu To Giang: Ikzf1 and Runx3 transcription factors negatively modulate the mesodermal development from pluripotent embryonic stem cells

Zsuzsanna Kolostyák: Examination of zymosan induced transcriptomic changes of Egr2 transcription factor deficient alveolar macrophages

Márta Mezei and Júlia Veres: Role of NLRP3inflammasomes in the antimicrobial activity of human plasmacytoid dendritic cells

Anna Rácz: High-throughput RNA sequencing revealed invasive aspergillosis specific (IA induced) miRNA signatures in the whole blood samples of onco-hematology patients

Alexandra Varga: Study the role of miR30 family members in ovarian cancer

Marcell Vas: Development of protease sensitive mouse pancreatic lipase

Viktória Vass and Viktória Nagy: Biochemical characterization of SARS-CoV-2 Mpro

Boglárka Ágnes Vinnai: Long-Term Thiamine Treatment Elevates Thermogenic Competency of Human Primary Neck and SGBS adipocytes

**11:15-12:35 Section 6 Chair: Tünde Fekete**

Link: https://unideb.webex.com/unideb/j.php?MTID=m6bae1ecb2e786de5982ae6812c758da4

***Introductory lecture***

11:15-11:35 Károly Jambrovics:
Arsenic trioxide (ATO) treatment together with all-trans retionic acid (ATRA) attenuates the cell survival potential of acute promyelocytic cells transglutaminase 2 (TG2)-dependent manner

***Regular talks***

11:35-11:50 Zsuzsa Csobán-Szabó:
An attempt to reveal the biological function of human transglutaminase 4 in the human saliva

11:50-12:05 Ádám Diós:
Serum reaction of celiac disease patients to gliadin peptides p31-43 and p57-68 arise from deamidation independent cross-reactive γ-gliadin specific antibodies

12:05-12:20 Vanda Toldi:
Misfolding of pancreatic lipase (PNLIP) mutants leads to endoplasmic reticulum stress and pancreatitis

12:20-12:35 Márió Miczi:
Development of a bio-layer interferometry-based protease assay

**12:35-13:15 Lunch**

**13:15-14:45 Section 7 Chair: Krisztina Köröskényi**

Link: https://unideb.webex.com/unideb/j.php?MTID=mc873c3a70296ef50a98f2b714cb0f95c

***Regular talks***

13:15-13:30 Tamás Linkner:
Analysis of changes in the cellular proteome and transcriptome following HIV infection and the role of HIV-2 Vpx

13:30-13:45 Erdenetsetseg Nokhoijav:
Evaluation of serum sample processing methods for metabolomics and proteomics analyses

13:45-14:00 Balázs Kunkli:
Annotation pipeline of grape and wine metabolites detected by High-Resolution Mass Spectrometry

14:00-14:15 Zsuzsanna Gyöngy:
Investigation of the catalytic cycle of ABCG2 in permeabilized cells

14:15-14:30 Kuljeet Singh:
The effects of sour cherry flavonoids and anthocyanins on Pgp activity

14:30-14:45 Éva Fige:
Heme oxygenase-1 in efferocytosis

**14:45-15:15 Break**

**15:15-16:15 Section 8 Chair: Kitti Pázmándi**
Link: https://unideb.webex.com/unideb/j.php?MTID=mdf0e627f23f9b7465851abe2e43d0cc6

15:15-15:30 Andrea Tóth:
Daprodustat accelerates elevated phosphate-induced osteogenic trans-differentiation of vascular smooth muscle cells and aorta calcification

15:30-15:45 Hajnalka Emese Halász:
The role of neutrophil granulocytes in skeletal muscle inflammation and regeneration

15:45-16:00 Nour Al-Zaeed:
Investigating the role of TAM kinase signaling in muscle regeneration

16:00-16:15 Nastaran Tarban:
Role of retinol saturase enzyme in skeletal muscle regeneration

**16:15 Concluding remarks** József Tőzsér (Chair)
Gábor Szabó
László Fésüs
István Balog
Szilvia Benkő
Bálint Nagy
Zsuzsa Szondy

# ABSTRACTS

## Section 1

### The linkage of genomic stability to breast cancer chemoprevention by rexinoid-based drug combinations

Sham Jdeed[1], Edina Erdős, Bálint L. Bálint[2], Iván P.Uray[1]
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[2]*Department of Biochemistry and Molecular Biology, University of Debrecen, Hungary*
*Keywords: cancer prevention, rexinoids, breast cancer, genomic stability, SWI/SNF*

Previously we determined that the RXR selective retinoid bexarotene and carvedilol, a non-selective beta-blocker represent a combination that is synergistic to suppress cell proliferation and suppressed Her2-induced breast cancer formation in a hormone-independent way, at low doses. However, the mechanisms behind their effect are yet to be elucidated. Proteomic data (RPPA) showed increased protein levels of ARID1A, a SWI/SNF subunit, in primary human mammary epithelial breast cells (HME-hTert) treated with the combination of bexarotene and carvedilol (B+C). ARID1A is a tumor suppressor that is mutated in 8% of breast cancer patients [1].

We hypothesized that the elevated level of ARID1A alters nucleosome organization to modulate the expression of genes related to genomic stability and proliferation. Our goal was to identify the role of chromatin remodeling behind the anti-proliferative effects of B+C through the actions of ARID1A. RPPA results were validated with Western-blot and Immunocytochemistry. ARID1A protein and transcript levels were studied after B+C treatment for different time points. ARID1A binding events after B+C treatment were identified through chromatin immunoprecipitation followed by sequencing (ChIP-Seq) and the results were validated with ChIP-qPCR. HME-hTert and MCF-7 breast cell lines were used and the latter was considered a model for ChIP assay optimization. ChIP-Seq results on HME-hTert and MCF-7 cells showed that ARID1A mainly occupied genomic regions distal to the transcription start site in correlation with H3K27ac marks. In addition, we found that about 80% and 60% of ARID1A binding sites in the B+C treated samples were unique compared to the control in HME-hTert and MCF-7 cells, respectively. We detected an enrichment of ARID1A along with H3K27ac marks on the regulatory regions of genes related to DNA damage repair, differentiation and proliferation regulation. Moreover, in order to select ARID1A candidate target genes we intersected ChIP-Seq and gene expression datasets and identified a number of genes related to cancer development including FOXQ1, KLF4 and BMP6.

We validated ARID1A and BRG1, the ATPase subunit in SWI/SNF, binding events to the selected regions through ChIP-qPCR. We could detect ARID1A enrichment to the potential regulatory region of FOXQ1 along with down-regulation of FOXQ1 gene expression levels upon B+C treatment in HMEC-hTert cells. FOXQ1 transcript levels increased significantly after ARID1A knock down. The data suggest that ARID1A regulates and suppresses the expression of the cancer promoting gene FOXQ1 upon B+C treatment.

***References:***

*1. Gao et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci. Signal.*
*2013.Apr.Availablefrom:https://www.cbioportal.org/results/cancerTypeSummary?cancer_study_list=5c8a7d55e4b046111Fee2296&case_set_id=all&gene_list=ARID1A.*

**The role of histone modifying proteins in double-strand DNA breaks in meiotic *Saccharomyces cerevisiae* cells**

Csaba Fillér[1], Beáta Boros-Oláh[1], Dóra Szabóné Varga[1], Adrienn Horváth[1], Orsolya Feró[1], Lóránt Székvölgyi[1,2]

[1] MTA-DE Momentum, Genome Architecture and Recombination Research Group, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Hungary
[2] Faculty of Pharmacy, University of Debrecen, Hungary

***Background:*** Histone H3 lysine 4 trimethylation (H3K4me3) is a key element for the initiation of meiotic recombination by marking meiotic DSB sites (3). DSBs occur during the first reductional division of meiosis at particular genomic sites called hotspots, which are located in intergenic regions in *Saccharomyces cerevisiae* (4). The evolutionary conserved Set1 and Spp1 are the members of the Set1C/COMPASS histone modification complex (4). Set1 is the histone methyltransferase (catalyzing the mono-, di-, and tri-methylation of histone H3 at lysine 4), while Spp1 regulates the tri-methylase activity of Set1 through a H3K4me3-reader PHD (plant homeodomain) finger domain and a Mer2-binder CxxC zinc finger motif (1, 2, 4). However, molecular details of the Spp1-mediated chromatin tethering process remain unknown.

***Method:*** We applied a high resolution chromosome conformation capture-based (3C) approach called Hi-C to investigate the spatial interaction of histone modifying proteins linked to the DSB process in meiotic yeast cells. Chromatin regions that interact with each other are fixed with formaldehyde. Hi-C allows to detect the all intra- and interchromosomal interaction in the genome. Our investigations were carried out on wild-type and mutants yeast strains at seven meiotic timepoints (0, 2-7h) after yeast cells were added to sporulation medium (SPM) for *spp1Δ*, *set1Δ* and *mer2sID* (sID: Spp1 interaction-deficient) mutants and at nine timepoints (0-8h) for *spp1ΔPHD* mutant. NGS sequencing was performed on two biological replicates in wild type cells and *spp1Δ*, *set1Δ* and *mer2sID* mutants, and one replica from *spp1ΔPHD* mutant. The depth of sequencing (~20 million reads per timepoint) allows for high spatial resolution and efficient bioinformatical analysis.

***Results:*** In the absence of functional Spp1 and Set1 proteins, H3K4me3- and DSB levels are greatly reduced (1, 4) and loop-axis tethering is expected to be affected. Therefore, we expect to identify a significant change in inter- and intrachromosomal spatial interactions at meiotic DSB sites, particularly in the prophase of meiosis when DSBs normally form.

Indeed, preliminary results obtained with PHD domain-mutant Spp1 showed a remarkable dysfunction in chromatin loop formation during meiosis, with decreased levels of intra- and interchromosomal (centromere-centromere) interactions.

1. Acquaviva, L., Székvölgyi, L., Dichtl, B., Dichtl, B.S., de La Roche Saint Andre,C., Nicolas, A., Geli, V.: The COMPASS subunit Spp1 links histone methylation to initiation of meiotic recombination. (2013) Science, 339 (6116), pp. 215-218.
2. Karányi, Z., Halász, L., Acquaviva, L., Jonas, D., Hetey, S., Boros-Oláh, B., Peng, F., Chen, D., Klein, F., Geli, V., Székvölgyi, L.: Nuclear dynamics of the Set1C subunit Spp1 prepares meiotic recombination sites for break formation. (2018) J Cell Biol, 217 (10), pp. 3398-3415.

3. Székvölgyi, L., and Nicolas, A.: From meiosis to postmeiotic events: homologous recombination is obligatory but flexible. (2010) FEBS J. 277, 571–589. doi: 10.1111/j.1742-4658.2009.07502.x
4. Zhang, Y., Suzuki, T., Li, K., Gothwal, S.K., Shinohara, M., Shinohara, A.: Genetic interactions of histone modification machinery Set1 and PAF1C with the recombination complex Rec114-Mer2-Mei4 in the formation of meiotic DNA double-strand breaks. (2020) Int J Mol Sci, 21 (8), pp. 2679. doi: 10.3390/ijms21082679.

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## Exploring new R-loop regulators by barcode RNA-DNA hybrid immunoprecipitation sequencing (BC-DRIP-seq)

Beáta Boros-Oláh[1], Zsolt Karányi[1], Éva Nagy[1], Adrienn Horváth[1], Dóra Szabóné Varga[1], Csaba Fillér[1], Lóránt Székvölgyi[1,2]

[1] MTA-DE Momentum, Genome Architecture and Recombination Research Group, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Hungary
[2] Faculty of Pharmacy, University of Debrecen, Hungary

**Background:** R-loops are three-stranded structures, which form when RNA hybridises to a complementary DNA strand, forming an RNA/DNA hybrid, resulting in displacement of the other DNA strand. R-loops have been shown to play an essential function in replication and during immunoglobulin class switch recombination and have been implicated in the control of gene expression. R-loop formation also affects the chromatin landscape of cells by inhibiting the DNA binding of chromatin regulatory factors, such as the Polycomb repressive complex 2. Not surprisingly, changing the physiological R-loop homeostasis can impair R-loop regulatory processes, which leads to case genome instability formation of human diseases.
**Objectives and Methods:** We aim to identify regulators of R-loop formation using a systematic approach called Barcode DRIP-seq, performed in a barcoded gene deletion library in the budding yeast S cerevisiae (involving ~4300 mutants). We measure endogenous R-loop levels near the barcoded region using an RNA-DNA hybrid specific antibody for R-loop enrichment, followed by next-gen sequencing.
**Results:** We identified several genes whose inactivation induced or eliminated R-loop structures at the barcoded test locus. We have found 59 genes with a clear mitochondrial function that showed significantly reduced R-loop levels, suggesting an unexpected role of mito-nuclear signalling processes in the homeostasis of chromosomal R-loops.
**Perspective:** We have started to characterize the significant hits revealed by the BC-DRIP screen by using whole-genome DRIP-seq to identify potential global changes of R-loop levels. In addition, we are going to perform functional studies in the mitochondrial mutants showing significantly increased nuclear R-loop levels. In conclusion, our high-throughput screen is expected to lead to a better understanding of the genetic architecture of R-loops formation.

**Acknowledgements.** This work was funded by NKFIH-NNE-130913, GINOP-2.3.2-15-2016-00024. L.Sz. was supported by the Bolyai Janos fellowship of the Hungarian Academy of Sciences and the UNKP-20-5-DE-47 new national excellence program of the Ministry For Innovation and Technology from the source of the National Research, Development and Innovation Fund.

**Estrogen mediated expression of miR200 family members in ovarian cells**

Éva Márton[1], Alexandra Varga[1], Dóra Domoszlai[1], Gréta Kiss[1], András Penyige[1,2], Bálint Nagy[1], Melinda Szilágyi-Bónizs[1]

[1]Department of Human Genetics, Faculty of Medicine, University of Debrecen

[2] Faculty of Pharmacology, University of Debrecen

Introduction: The exposure to estrogens increases the risk for gynecological cancers including ovarian cancer. Accumulating evidence suggest that this effect is also exerted by xenoestrogens that are natural (e.g. zearalenon) or synthetic compounds (e.g. bisphenol A), which mimic the effect of physiological estrogens. In our previous studies estradiol, zearalenon and bisphenol A had proliferative and migrative effect on the Estrogen Receptor α (ERα) positive ovarian cell line (PEO1) that was not observed in the lack of this receptor (in A2780). The aim of our study was to investigate the effect of ERα and estrogens to the expression of miR200 family members that are considered to be tumor supressors and involved in the regulation of cell cycle and invasiveness of cancer.

Materials and methods: We applied the PEO1 and A2780 human epithelial ovarian cell lines in our studies. The intracellular and cell-free expression of miR200a-3p, miR200b-3p, miR200c-3p, miR141-3p and miR429-3p was studied by qPCR. When the effect of estrogens was studied the PEO1 cells were treated with 10 nM estradiol, zearalenon and bisphenol A. MPP was applied as an ERα selective antagonist.

Results: The basal expression of miR200s was significantly higher in the ERα positive PEO1 cell line than in A2780 that was observed in the case of the intracellular and cell-free expression levels as well. MiR200b and miR200c possessed the highest expression level that suggests their high biological relevance. When PEO1 cells were treated with estrogens the up-regulation of miR200s was observed in the cell lysates 12 h after the treatments that was followed by their down-regulation later compared to the non-treated control. This effect was higher in response to estradiol treatment than to zearalenon or bisphenol A, which molecules have lower affinity to ERα. The cell-free expression of these miRNAs was also altered by estrogen exposure, however, these alterations were not so significant as in the cell lysates. When cells were treated with an ERα selective antagonist together with estradiol the inductive effect of estradiol to the expression of miR200s was significantly decreased.

Conclusion: We conclude that cell-free miR200s might be applicable biomarkers for estrogen sensitivity of ovarian cells that support therapy selection in ovarian cancer (e.g. the application of estrogen blocking agents such as tamoxifen).

# Section 2

### Batokines – impact on adipocyte browning and metabolic homeostasis

Endre Kristóf[1], Attila Vámos[1], Abhirup Shaw[1], Rini Arianti[1], Boglárka Ágnes Vinnai[1], Krisztina Ajna Chalupa[1], Ferenc Győry[2], Szilárd Póliska[1], Beáta B. Tóth[1], László Fésüs[1]
Departments of Biochemistry and Molecular Biology[1] and Surgery[2], Faculty of Medicine, University of Debrecen

Brown adipose tissue (BAT) plays a major role in maintaining the constant core body temperature of humans, without shivering. Active BAT can be found in specific adipose depots in adult humans, amount to 1.5% of total body mass and are mostly enriched in the supraclavicular, deep neck (DN) and paravertebral regions. Brown and beige adipocytes accumulate numerous small lipid droplets in a multilocular arrangement, contain a large amount of mitochondria-rich cytoplasm and convert glucose and fatty acids into heat. The heat production is mainly mediated by uncoupling protein 1, a mitochondrial carrier protein that uncouples ATP synthesis from the respiratory chain activity. The ratio of 'anti-obesity' beige and 'pro-obesity' white precursors and adipocytes is strongly influenced by genetic predisposition; FTO rs1421085 T-to-C single nucleotide polymorphism shifts differentiation program towards white adipocytes in the subcutaneous fat. In parallel, an important secretory role of BAT was revealed in the recent years leading to an increased interest in identifying "batokines" that can exert autocrine (resulting in ether positive or negative regulation of thermogenesis), paracrine (targeting sympathetic nerve endings, vascular or immune cells) or endocrine (allowing for crosstalk between the BAT and other organs, including the liver, cardiac and skeletal muscle) effects. In addition, subpopulations of adipocytes within distinct adipose tissue depots can have a unique secretory phenotype that determines their regulatory role in a heterogeneous tissue environment.
To learn more about human adipocyte browning, we studied global gene expression patterns of white and brown (in response to sustained PPARγ stimulation) differentiated neck adipocyte populations derived from primary human adipose-derived stromal cells. Several genes and gene expression-related pathways encoding or involving secreted proteins were found as a part of the pattern of gene expression regulated by the presence of the obesity-risk allele of the FTO locus, the anatomical origin of the progenitors or PPARγ-induced browning differentiation.
Next, we aimed to investigate selected cyto- or chemokines, based on our recently reported RNASeq data and literature mining, at protein level. The presence of IL-6, fractalkine, S100b, CXCL1 and 2 was detected by ELISA from subcutaneous and DN tissue supernatants. Except for CXCL2, DN adipose tissue released more of the investigated factors than the subcutaneous ones). However, out of those only the well-defined "batokine", IL-6 was present in the conditioned media of *ex vivo* differentiating adipocytes. Further research is needed to clarify the exact source and functions of the proposed "batokines" in humans.
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**Thiamine Enhances Thermogenic Activation in Human Neck Adipocytes**

Rini Arianti[1,2], Boglárka Ágnes Vinnai[1], Abhirup Shaw[1,2], Attila Vámos[1,2], Ferenc Győry[3], Endre Kristóf[1], László Fésüs[1]

[1] Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen
[2] Doctoral School of Molecular Cell and Immune Biology, University of Debrecen
[3] Department of Surgery, Faculty of Medicine, University of Debrecen

Activation of non-shivering thermogenesis by brown adipose tissue (BAT) has been suggested as one of the attractive approaches to treat obesity and related metabolic disturbance as it augments energy expenditure. Human BAT is interspersed in several anatomical regions including cervical (deep neck), supraclavicular and mediastinal. Recently, we used high throughput RNA sequencing technology to analyze global gene expression pattern of *ex vivo* differentiated subcutaneous (SC) and deep neck (DN) adipocytes and found 1049 differentially expressed genes when the adipocytes of different origins were compared. Out of the 1049 genes, we found 21 cell membrane solute carrier (SLC) transporters which play role in various biological processes. We selected thiamine transporter 2 (ThTr2) for further investigation.
We added excess amount of thiamine to the cell culture media of differentiated human primary SC and DN adipocytes. The mRNA and protein expression of ThTr2 and uncoupling protein-1 (UCP1) were affected by the short-term thiamine treatment. In addition, functional analysis revealed an elevation of oxygen consumption rate (OCR) in cAMP activated, white-differentiated SC and DN adipocytes upon short-term thiamine treatment. Further, we inhibited ThTr2 activity in white-differentiated SC and DN adipocytes using a ThTr2-specific inhibitor fedratinib, which attenuated cAMP- and thiamine-stimulated UCP1 upregulation at mRNA and protein level. cAMP-stimulated OCR was also reduced by fedratinib. Oxythiamine, the inhibitor of mitochondrial thiamine pyrophosphate-dependent enzymes, did not decrease UCP1 expression and OCR.
Our *ex vivo* study suggests a novel opportunity to increase thermogenic activation of human browning adipocytes by revealing a so far not recognized importance of ThTr2 in the thermogenic response of human SC and DN adipocytes.

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**Irisin treatment stimulated the release of CXCL1 but prevented the upregulation of thermogenesis in human primary cervical subcutaneous and deep neck adipocytes**

Abhirup Shaw[1], Beáta B Tóth[1], Rini Arianti[1], Attila Vámos[1], Ferenc Győry[2], Szilárd Póliska[1], László Fésüs[1], Endre Kristóf[1]
Departments of Biochemistry and Molecular Biology[1] and Surgery[2], Faculty of Medicine, University of Debrecen

PET/CT verified the presence of uncoupling protein (UCP1) dependent thermogenic brown adipose tissue (BAT) in healthy adult humans and highlighted the negative correlation between obesity and BAT amount. These thermogenic fat depots are enriched in “deep neck” (DN) regions, but it has not been clarified how they respond to different natural browning-inducers. Irisin was discovered as a myokine that drives beige adipogenesis in murine subcutaneous white adipose tissue (Boström P. et al., 2012, Nature). We found that irisin

could induce the beige differentiation program in human primary subcutaneous and SGBS white adipocytes *ex vivo* (Kristóf E. et al., 2015, Sci Rep; Klusóczki Á. et al., 2019, Sci Rep). In this study, we intended to characterize how white differentiated adipocytes from DN and cervical subcutaneous (SC) human adipose tissue depots responded to a continuous irisin administration.
Preadipocytes obtained from SC and DN regions of 9 donors were differentiated into adipocytes in the presence or absence of irisin at 250 ng/mL concentration. Cell lysates and conditioned media were collected; global gene and protein expression analysis was performed by RNA-sequencing and immunoblotting. The presence of fractalkine, IL-32, CXCL1 and 2 were detected by sandwich-ELISA from conditioned differentiation media. Oxygen consumption and extracellular acidification were measured using an XF96 oxymeter.
Irisin treatment resulted in upregulation of 50 and 66 genes in SC and DN adipocytes, whereas 2 and 7 genes were downregulated, respectively. Genes involved in cytokine and interferon signaling and of several chemokines were upregulated as a result of irisin treatment. CXCL1, a small peptide which belongs to the CXC chemokine family that acts as a chemoattractant for several immune cells or other non-hematopoietic cells, was found to be the highest upregulated gene upon irisin treatment, which was further validated by RT-qPCR. ELISA results showed that CXCL1 was released into the media (>1000 pg/mL) throughout the differentiation period, only by the irisin treated cells. Removal of irisin after a 14 days long differentiation program abolished the secretion of CXCL1. The most likely cause of the CXCL1 release is the upregulation of the NFκB pathway. Fractalkine, IL-32 and CXCL2 were not secreted by the differentiating adipocytes, although Irisin strongly upregulated their gene expression.
Surprisingly, irisin treatment prevented the upregulation UCP1 gene and protein expression both in SC and DN adipocytes differentiated *ex vivo*. The expression of OXPHOS protein complexes also followed a similar pattern. cAMP stimulated and proton leak respiration were less induced when the adipocytes were differentiated in the presence of irisin, especially in the DN samples. Further research is required to reveal the underlying molecular mechanisms of the adipose tissue depot-specific effects of irisin in humans.
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**Systematic analysis of beige to white transition of human primary subcutaneous adipocytes**

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We distinguish three types of adipocytes: the energy storing white, and the two thermogenic classical brown and beige adipocytes. Both heat producing adipocytes have multilocular lipid droplets, increased mitochondrial mass and express high level of uncoupling protein 1 (UCP1); furthermore they promote energy expenditure, secrete specific cytokines (batokines) and metabolites and can be potential targets for treating metabolic disorders and obesity. When the thermogenic stimulus subsides, selective autophagy is activated for mitochondrial clearance (mitophagy) and inactive beige adipocytes persist that, however, have a white adipocyte-like morphology *in vivo*.
In this study, we investigated this beige to white conversion in primary human subcutaneous abdominal adipocytes with different pro-obesity FTO rs1421085 genotypes. Next we aim to find an optimal condition to study the process with a system biology approach.

Human primary subcutaneous preadipocytes were differentiated to beige adipocytes for 14 days, and then treated with dibutyryl-cAMP for 4 hours. Then, either the same culture conditions were applied for additional 14 days (active beige adipocytes) or it was replaced by a white differentiation medium (inactive beige adipocytes). As a negative control, white adipocytes were differentiated by a specific cocktail. Cell lysates and conditioned media were collected for systematic transcriptomic, proteomic and metabolomic analyses.
The long-term (28 days) beige differentiation, driven by the PPARγ agonist rosiglitazone, resulted in increased UCP1 gene and protein expression as compared to the short-term (14 days) protocol. cAMP caused further increase in the gene expression levels of several browning markers (UCP1, CIDEA and PGC1a), however, did not affect CKMT1 and 2 expression. After mid-term cAMP stimuli (4 h long treatment at day 14), the thermogenic competency of beige adipocytes was maintained or even enhanced by utilizing the beige protocol. However, when we applied the white protocol after day 14, the beige to white conversion was observed in these samples as well. Only the conditioned media of the active beige adipocytes contained the batokine IL-6. Active beige adipocytes consumed more cysteine but less aspartate and glutamate then the white and inactive beige adipocytes. The applied *ex vivo* model enables us to analyze the global gene expression of adipocytes with different FTO allele status by RNA sequencing and their secretome by mass spectrometry in the near future.
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# Section 3

## Prior IL-4 exposure induces chromatin remodeling and the rearrangement of LPS-activated p65 cistrome, leading to synergistic transcriptional activation of a specific gene set in macrophages

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In response to various environmental signals or pathogen-derived molecules, macrophages undergo extensive phenotypic shift. The two extreme types are called classical or M(INF-γ) and alternative or M(IL-4) phenotype, but in complex tissue environments these cells often display a broad spectrum of macrophage polarization states. Using genome-wide technologies such as ChIP-seq, ATAC-seq and RNA-seq, we investigated the epigenetic and transcriptomic outcomes of inflammatory response in alternatively polarized mouse macrophages. Unexpectedly, we found that IL-4 pretreatment enhances the LPS-responsiveness of 941 genes including cytokines, chemokines, and several immune response-related genes in a STAT6 dependent manner. This phenomenon is associated with the IL-4-mediated remodeling of chromatin structure and the elevated LPS-activated NFκB-p65 binding at the regulatory regions of synergistically activated genes in alternatively polarized macrophages. Finally, both the enhanced LPS-responsiveness of this gene set and also elevated NFκB-p65 binding at the enhancers proved to be at least partially dependent on the presence of the IL-4-induced EGR2 transcription factor. Taken together, these findings suggest that the complex interaction between alternative polarization and inflammatory signals in macrophages is orchestrated at the epigenomic level and can lead to synergistic gene regulatory events.

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## INVESTIGATING THE INTERACTION OF NLRP3 AND TYPE I INTERFERON PATHWAYS IN HUMAN PLASMACYTOID DENDRITIC CELLS

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**Introduction:** Generally reciprocal antagonistic effects can be observed between the antiviral type I interferon (IFN) and the antibacterial IL-1β pathways, which not only affect antimicrobial responses, but also shape the immune responses in autoimmune diseases. Plasmacytoid dendritic cells (pDCs) as professional type I IFN producing cells are the major coordinators of antiviral immune responses. However, the NLRP3-dependent IL-1β secretory pathway in pDCs is still unexplored. Thus, our aim was to investigate the functional activity of the IL-1β pathway in human pDCs and to examine the possible interactions between type I IFN and IL-1β pathways.
**Methods:** Human pDCs were treated with distinct Toll-like receptor (TLR) agonists, which differ in their type I IFN inducing capacity, in the presence or absence of IFN-α cytokine, then the activity of the NLRP3 pathway was analyzed by Q-PCR, western blot, ELISA and flow cytometry.
**Results:** We observed that the applied TLR9 agonists induced pro-IL-1β production in pDCs in a different manner. CpG-A, which is a strong type I IFN inducer, promoted pro-IL-1β production in pDCs to a lesser extent than CpG-B, which is a strong activator of the NF-kappaB pathway, but results only in a low IFN-α release. Nigericin-elicited mature, cleaved IL-1β secretion could only be detected after CpG-B pre-treatment of pDCs. However, CpG-B treatment in the presence of IFN-α also resulted in lower IL-1β production in pDCs, suggesting that type I IFNs may inhibit NLRP3 inflammasome activity probably via activating the type I IFN dependent STAT1 signaling cascade.

**Conclusion:** Our results suggest that the NLRP3-dependent IL-1β secretory pathway is inducible in human pDCs. However, the IL-1β-mediated inflammatory response of pDCs can be inhibited by activating the type I IFN pathway.
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## MODULATION OF HUMAN DENDRITIC CELL MEDIATED INFLAMMATORY PROCESSES BY PHYTOCHEMICALS

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**Introduction:** Plants are a rich source of bioactive phytochemicals, the pharmacological and therapeutic properties of which have been extensively studied lately. Among them, the bioactive components of ginger are emerged as potent anti-inflammatory compounds, which are able to exert immunomodulatory effects on different immune cell types. Nevertheless, their effects and mechanism of action has not been explored in human dendritic cells (DCs) yet. In the present study, we evaluated how Toll-like receptor (TLR)-mediated responses of human DCs are affected by 6-gingerol and 6-shogaol, which are the major functional compounds of ginger.
**Methods:** Human monocyte-derived DCs (moDCs) were activated with the TLR4 agonist lipopolysaccharide (LPS) or live Escherichia coli (E. coli) in the presence or absence of 6-gingerol or 6-shogaol. Thereafter, the phenotypical and functional changes of the cells were monitored by flow cytometry, ELISA and western blotting.
**Results:** We found that the bioactive compounds of ginger significantly decreased the TLR4 activation-induced production of pro-inflammatory cytokines and suppressed the expression of costimulatory and activation markers in moDCs in a concentration dependent manner. The compounds of ginger also suppressed the cytokine producing ability of moDCs upon stimulation with live E.coli. Further, we found that 6-gingerol and 6-shogaol inhibited the TLR4 activation-mediated phosphorylation of IkBα and p65, the members of NF-kB signaling pathway and prevented the phosphorylation of p70S6 kinase and Akt, the major components of mammalian target of rapamycin (mTOR) signaling cascade.
**Conclusion:** Our results indicate that the major bioactive compounds of ginger interfered with the TLR4-mediated inflammatory responses of human moDCs, probably by inhibiting the NF-κB and mTOR signaling cascades, which are essential for a multitude of functions in DCs. Thus, our results demonstrate that the examined bioactive compounds of ginger exert potent anti-inflammatory and immunomodulatory effects on human DCs.
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## Egr2 transcription factor is a potential regulator of differentiation and pathogen elimination in alveolar macrophages

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Alveolar macrophages (AMs) are parts of the pulmonary mononuclear phagocyte system. As a component of innate immunity, AMs provide fundamental protection against air pollutants and pathogens. The essential role of AMs is well known in pulmonary pathologies. The examination of transcriptional processes which regulate the development and functions of AMs is important in the identification of therapeutic strategies.

Early Growth Response 2 (Egr2) is a transcription factor which fulfills major role in cellular differentiation but its complex epigenetic role in macrophages has not well described.

In our work, we used Egr2 LysozymeM-Cre mice for the examination of transcriptomic and epigenetic alterations upon the loss of Egr2 in AMs. AMs were isolated from lung using bronchoalveolar lavage. We performed RT-qPCR and RNA-sequencing to determine the changes in transcriptome and ATAC-sequencing to define chromatin openness. Based on the bioinformatically integrated results of genome-wide approaches, we predicted the potential functional outcomes. Our findings support an important role of Egr2 in transcriptional regulation of AMs. We described significant differences in mRNA level of 981 genes. Moreover, the chromatin openness shows large scale alterations between control and Egr2 deficient AMs. Our pathway prediction pointed out the potential impairment of pathogen engulfment in the loss of Egr2 and we successfully demonstrated the functional differences in phagocytosis. The phagocytotic activity of AMs was determined by fluorescently labeled zymosan bioparticle assays.

Our findings indicate the transcriptional regulatory role of Egr2 in AMs and we identified this factor as a potential key director of pathogen elimination associated pathways impacting phagocytosis.

**Investigation of complex interactions between alternative macrophage polarization signals and HIF-1α signaling pathway**

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As an essential cellular component of the innate immune system, macrophages play a crucial role in various physiological and pathological conditions. The functional heterogeneity and plasticity of macrophages are regulated at transcriptional and post-transcriptional levels. Different cell activation signals are capable of transiently modifying the activity of macrophages, already bearing a given identity. One out of extreme endpoints in microenvironmental signals-induced macrophage polarization states is IL-4-STAT6 signaling pathway-activated alternative (M2) macrophage polarization. This polarization state is characterized by highly active oxidative phosphorylation, anti-inflammatory properties, and tissue regeneration capacity. O2 level can be very different and change dynamically in various tissue. In pathological hypoxia, the Hypoxia-inducible factor-1α (HIF-1α) transcription factor is crucial for cellular adaptation when O2 availability is limited for energy production. Although M2 macrophage polarization is observed in various hypoxia-linked pathological conditions, including tumors and Th2-type airway inflammation and asthma, the interactions between hypoxia and M2 polarization signals-activated pathways are less studied.
Here we aimed to investigate how IL-4-induced M2 macrophage polarization and hypoxia-mediated HIF-1α stabilization interact at gene expression level in murine bone marrow-derived macrophages (BMDM) model system. For the stabilization of HIF-1α, we applied a generally accepted hypoxia mimetic agent CoCl2 (50μM) or hypoxia chamber (1%O2). We determined the gene expression changes following 24 hours IL-4 and CoCl2 (or hypoxia) co-treatment using RNA sequencing and RT-qPCR.
We found that several alternative macrophage polarization markers such as Arg1 and Chil3 and tissue-remodeling factors, including Mmp12 and Mmp13 showed elevated IL-4 response in the presence of CoCl2 or hypoxia. In contrast, hypoxia-inducible Vegfa expression was repressed in both normoxic and hypoxic conditions by IL-4.
Taken together, our results suggest a complex interaction between IL-4-STAT6 and HIF-1α signaling pathways in macrophages. However, the molecular bases and functional consequences require further investigations.

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**All-Trans Retinoic Acid Enhances both the Signaling for Priming and the Glycolysis for Activation of NLRP3 Inflammasome in Human Macrophage**

Ahmad Alatshan, Gergo E. Kovács, Azzam Aladdin, Zsolt Czimmerer, Krisztina Tar and Szilvia Benko

-trans retinoic acid (ATRA) is a derivative of vitamin A that has many important biological functions, including the modulation of immune responses. ATRA actions are mediated through the retinoic acid receptor that functions as a nuclear receptor, either regulating gene transcription in the nucleus or modulating signal transduction in the cytoplasm. NLRP3 inflammasome is a multiprotein complex that is activated by a huge variety of stimuli, including pathogen- or danger-related molecules. Activation of the inflammasome is required for the production of IL-1β, which drives the inflammatory responses of infectious or non-infectious sterile inflammation. Here, we showed that ATRA prolongs the expression of IL-6 and IL-1β following LPS activation in human monocyte-derived macrophages. We describe for the first time that ATRA modulates both priming and activation signals required for NLRP3 inflammasome function. ATRA alone induces NLRP3 expression, and enhances LPS-induced expression of NLRP3 and pro-IL-1β via the regulation of several signal transduction pathways. We show that ATRA alleviates the negative feedback loop effect of IL-10 anti-inflammatory cytokine on NLRP3 inflammasome function by inhibiting the Akt-mTOR-STAT3 signaling axis. We also provide evidence that ATRA modulates the DRP1(Mitochondrial fission protein) and hexokinase 2 expression, and shifts the metabolism of LPS-activated macrophages toward glycolysis, leading to the activation of NLRP3 inflammasome.

# Section 4

**Fat transplantation restores metabolism of fatty liver in PPARγ deficient mouse model.**

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The most important role of adipose tissue is to store energy, protect the body from physical effects and produce leptin and adiponectin as endocrine organs.
Here, we present data using a new fat deficient mouse model which lacks peroxisome proliferator-activated receptor gamma (PPARγ), a transcription factor which plays a crucial role in adipogenesis and lipid metabolism. To circumvent the embryonic lethality of its germline deletion we generated Sox2Cre-Pparγ(floxed) mice which could survive but had phenotypic abnormalities, high newborn lethality and high sensitivity to environmental

conditions (temperature, humidity). Normal laboratory conditions create challenges in survival, breeding and experimental design in adipose tissue-deficient mouse models. We could improve the survival and the colony size by keeping mice on thermoneutral temperature (30$^{o}$C) that allowed further study of their phenotype. We find that PPARγ KO mice suffered liver steatosis, organomegaly, insulin resistance and completely lacked white and brown adipose tissues. Calorimetric and metabolic parameter measurements with the Oxymax-CLAMS system showed that PPARγ KO mice had higher O2 and CO2 production, higher food and water intake but showed lower overall activity. Seeing the fatty liver, the question arises to how other tissues can compensate for the lack of adipose tissue. In our study, we focused on the other two major metabolic organs: the liver and muscle. The transcripomic analysis of the two organs showed altered expression of some fatty acid metabolism genes. In addition, lipidomic analysis showed an inverse correlation of free fatty acid, triglyceride, sphingomyelin, phosphatidilcholine and phosphatidilethanolamine acummulation in the liver and the muscle, respectively, in PPARγ KO mice. Finally, subcutaneous fat tissue transplantation into PPARγ KO mice could improve metabolic parameters, insulin sensitivity and decrease lipid accumulation in the liver. The distribution of different lipids in the liver of transplanted mice becomes similar to the controls. The PPARγ KO mouse line is a good model to investigate lipoatrophy and the regulatory role of adipose tissue in the body.

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**Tissue transglutaminase knock-out preadipocytes and beige cells of epidydimal fat origin possess lower mitochondrial functions**

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It has been discovered that cold exposure or adrenergic stimuli can provoke the appearance of clusters of uncoupling protein1-(UCP1)-positive heat-producing cells in white adipose tissue (WAT) through the process of browning. These inducible cells have been named ‘beige’ adipocytes and have an overlapping, but distinct gene expression pattern compared to classical brown adipocytes. To investigate the possible role of TG2 in browning mechanisms dependent on mitochondrial functions, first, we cold exposed mice and found that TG2-/- animals had decreased cold tolerance due to browning deficiency and low-fat mobilization in the epidydimal WAT (Madi, 2017). Then, we isolated preadipocytes from epididymal fat and differentiated them to beige direction. We compared beige cells treated with different adrenergic agonists and found that the differences between TG +/+ and TG2 -/- cells were not related to altered responses to adrenergic treatments, but they already existed in differentiated beige cells without adrenergic treatments. We found that protein expression of UCP1 and mitochondrial complex proteins were significantly lower in TG2 -/- beige cells, similarly to decreased mitochondrial DNA content and the NADH dehydrogenase activity. High Content Screening (HCS) results together with Western-blots on mitochondrial fission-fusion proteins suggest that the tubular mitochondrial morphology is characteristic of both cell types; however, there are significantly higher fractions of fragmented mitochondria in TG2-/- beige

cells compared to TG2 +/+ ones. Our results obtained from the LSC/Mitotracker and Seahorse measurements, together with detection of ATP, NADH and ROS production, suggest that in the absence of TG2 mitochondrial functions can be altered leading to lower heat generation capacity of TG2-/- beige adipocytes. We have also found that the expression of the well-known coactivator PGC1alpha protein regulating mitochondrial biogenesis is significantly lower in TG2-/- beige cells.
To learn more about the possible explanations of the obtained results, we have carried out RNA-seq experiments comparing gene expression profiles of TG2-/- and TG2+/+ cells. After validating the results using qPCR, we have found that the gene expressions of a mitochondrial acyl-carnitine transporter and a CoA transporter were significantly lower in TG2-/- beige cells. We are still validating the results about 20 other genes which might have significant roles in the mechanism and regulation of thermogenesis in beige adipocytes.

The work is supported by the ÚNKP-20-3 New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund, GINOP-2.3.2-15-2016-00006, and by the Hungarian Research Fund [OTKA-NK129139] projects. The project is co-financed by the European Union and the European Regional Development Fund.

# Establishing of the mutational spectrum of Hungarian patients with familial hypercholesterolemia

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**Background:** Familial hypercholesterolemia (FH) is a relatively common inherited metabolic disorder characterized by significantly elevated LDL cholesterol levels and increased thrombotic risk. In most cases the inheritance is dominant, rarely co-dominant. Several genes have been associated with FH. The diagnosis of FH is primarily clinical and based on laboratory parameters. Genetic testing may help identify personalized treatment strategies.
**Patients and methods:** 149 probands were tested for mutations in *LDLR*, *LDLRAP1*, *ApoB*, *PCSK9* and *STAP1* genes using next generation sequencing on Illumina MiSeq sequencer. Mutations were classified by standard criteria using population, segregation and *in silico* data. Detected mutations were confirmed by Sanger sequencing.
**Results:** Altogether 45 pathogenic or likely pathogenic (class 5/4) and 2 VUS (variant of unknown significance, class 3) variants were identified in 44/149 unrelated patients. 40 heterozygous mutations were detected in the *LDLR* gene in 37 patients. In 7 patients heterozygous *ApoB* mutations were detected. 4 of 47 mutations were novel. Three patients had 2 heterozygous *LDLR* mutations. More than half of the mutations (26/47) were missense. 5 nonsense mutations, 8 splicing mutations, 4 frameshift mutations, 1 in-frame deletion, 2 large deletions and 1 large duplication were also detected.
**Conclusions:** Our study provide data on the mutation spectrum for FH in Hungarian patients and confirms the genetic heterogeneity of the disease.
This study was supported by Ministry of National Economy, Hungary (GINOP-2.3.2-15-2016-00039).

**The role of lipid presentation by CD1c in cancer**

Rita Szőke-Kovács, Péter Gogolák

Cytotoxic T lymphocytes are some of the main players of the antitumor immune response in the body. These cells have been proved to be effective in targeted cancer therapy, however their application is limited by the phenomenon called allotype restriction caused by the extreme polymorphism of "classical" Major Histocompatibility Complex (MHC) molecules. Whereas the different alleles of the "classical" MHC molecules present tumour-associated peptide antigens for specific T cell recognition, the "non-classical" MHC-like molecules such as the Cluster of Differentiation 1 (CD1) molecules, belonging to the non-polymorphic MHC class Ib group, can present microbial lipids and self-lipids to specific T cells. The CD1 type c (CD1c) molecules were described to present lipid molecules that predominantly accumulate in leukaemia cells, so specific CD1c restricted T cells can mediate the killing of these leukaemia cells. Several other malignant cell types also express CD1c molecules, therefore these molecules can be regarded as targetable tumour markers. The non-polymorphic nature of these molecules renders them ideal therapeutic target for patients of different genetic background. Whether the CD1c molecules are present in all types of leukaemia and the mechanism of CD1c recognition by T cells is currently unknown, limiting our ability to generate CD1c-targeting biopharmaceuticals. Therefore, we aim to dissect the lipid antigen repertoire associated with CD1c complexes in macrophages, dendritic cells and cancer cells which may uncover possible cancer lipid targets. We also aim to investigate different blood born malignancies for the presence of CD1c and determine how it is recognised by T-cells. These findings will allow the development of a new generation of CD1c-targeting reagents specific to these malignancies and suitable across the whole human population by overcoming genetic restriction.

**Activation of Nrf2/HO-1/ferritin system attenuates high phosphate-induced calcification of valve interstitial cells**

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**Introduction:** Calcific aortic valve stenosis (CAVS) is a heart disease characterized by a progressive fibro-calcific remodelling and thickening of the aortic valves eventually leading to severe heart outflow tract obstruction. CAVS is the second most frequent cardiovascular disease with a prevalence of 0.4% in the general population and 1.7% in the population over 65 years old. Patients with chronic kidney disease (CKD) are characterized by accelerated and premature cardiovascular calcification and the prevalence of severe CAVS ranges between 6-13%. CAVS is an actively regulated process with the involvement of the differentiation of valvular interstitial cells (VICs) into osteoblast-like cells and myofibroblasts. CKD is associated with dysregulation of calcium (Ca) and phosphate (P) metabolism, and increased P and Ca synergistically stimulate osteogenic differentiation of VICs. Elevation of reactive oxygen species (ROS) formation play a role in valve calcification. Normally ROS formation is counterbalanced by a complex antioxidant defence system. Nuclear factor erythroid 2-related factor 2 (Nrf2) is an important element of this antioxidant network as it controls the

expression of a variety of antioxidant response element-dependent genes. Our aim was to test whether upregulation of the Nrf2 pathway by heme influences P and Ca-induced calcification of VICs, and to address the role of heme degradation products (bilirubin, Fe, CO) in the observed effects.

**Methods:** Osteogenic differentiation of human VICs was triggered by P (2.5 mmol/L) and Ca (0.3 mmol/L) in the presence or absence of heme bilirubin, Fe and CO releasing molecule (CORM) at different doses between 5 and 50 μmol/L. Extracellular matrix (ECM) calcification was determined by Alizarin red staining and calcium assay. Protein expressions were evaluated via western blot while mRNA levels were determined by quantitative real-time PCR.

**Results:** Heme decreased P-mediated calcification of VICs and, as expected, increased the mRNA and protein expressions of Nrf2 and heme oxygenase (HO-1). Heme lost its protective effect against VIC calcification upon inhibition of either Nrf2 or HO-1 enzyme activity. All the heme degradation products (bilirubin, Fe, CO) inhibited P-induced VIC calcification to a different extent. Moreover, ferritin, – upregulated by labile iron released from heme during HO-1-mediated degradation – also showed a strong inhibitory effect on P-induced VIC calcification.

**Conclusions:** We concluded that heme-mediated activation of the Nrf2/HO-1/ferritin axis protects against VIC calcification. We identified products of heme catabolism, bilirubin, Fe and CO, as well as ferritin as potent anti-calcification molecules.

## Clearance of apoptotic cells in obesity

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Increasing evidence indicate that impaired clearance of apoptotic cells contributes to the development of various chronic inflammatory diseases. Obesity is also an inflammatory disease and is often accompanied with other chronic inflammatory diseases. Free fatty acids (FFA) concentrations are usually elevated in obesity because the enlarged adipose tissue mass releases more FFA and macrophages contribute to the clearance of excess amount of FFA. In our experiments we are investigating whether exposure of macrophages to palmitate (a common dietary FFA) could affect apoptotic cell clearance efficiency of macrophages and thus form a link between obesity and the development of associated chronic inflammatory diseases.

Our results show that palmitate-treated macrophages have a significantly reduced capacity to phagocytose apoptotic cells, however palmitate has no significant effect on expression levels of phagocytic receptors. In our experiments we altered the AMPK-mTOR cellular energy-sensing signaling pathways. Based on our findings activation of AMPK by AICAR in macrophages increases their phagocytic capacity following palmitate treatment. Inhibition of mTOR signaling pathway improves the defective efferocytosis of macrophages triggered by palmitate treatment. ROCK inhibition by Y-27632 in macrophages effectively enhances their efferocytosis following palmitate treatment.

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# Section 5

### Genomics meets Genetics: A combined RNA-seq and WGS analysis at a 10-member rabbit family proves that the expression level at many genes shows intermediate inheritance and these expression differences might be caused by transcription factor binding site variations

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During a 'usual' differential gene expression analysis, we compare transcript abundances between different conditions. In these cases, we assume that the differences detected are due to the result of the gene expression regulation changes caused by the altered conditions. But what is happening when we detect gene expression differences between different individuals? Opposite to the previous example, here we may hypothesize that the differential expression is caused by genetic differences. Namely, the two individuals can have different sequences at the transcription factor binding sites, which in turn can result in a different affinity between the transcription factors and their cognate binding sites.
Our hypothesis is that if we consider a given gene, which is on an autosomal chromosome, the detected expression level is the sum of the expression from the maternal and paternal chromosomes. Therefore, if the expression levels originating from the maternal and paternal chromosomes are different, the inheritance of the cumulated expression levels of the given gene at the given condition, will follow a non-Mendelian inheritance pattern. Namely, taken the gene expression level as a trait, we will see a kind of intermediate expression inheritance.
To test this hypothesis, we have designed the following experimental setup: we take a rabbit family with eight children, where the mother was from a meat producing line, while the father was a dwarf hobby rabbit. We had taken 4-4 muscle samples from each of the ten individuals and Illumina sequenced both the transcriptomes and the whole genomes.
After finishing the usual RNA-seq and WGS analysis pipelines, we have started to examine those genes, where there were expression differences between the individuals. Surprisingly, we found perfect examples for each possible expression level combinations between the father and mother (AAxBB, ABxAB, ABxAA, ABxBB, where A is representing the higher expressing, while B is representing the lower expressing allele). For example, if the mother showed high while the father showed lower level of expression, the eight children either showed uniformly intermediate level of expression (AB), or the similar levels of expression as the parents (AA and AB or AB and BB). Here, we have started to utilize the genetic differences between the father and mother. Not surprisingly, we found the very same

genotype patterns at each example as were the inferred expression patterns. In addition, by scrutinizing the allele specific expression we could distinguish without doubt between each case. In the next step, we transformed the positions of our human consensus motif sets (Czipa et al 2020) into the rabbit reference genome. Due to the whole genome sequencing of every animal in the experiment, we could look for the genotypes of the conserved motifs around these genes at each individual. Strikingly, we could find perfect examples, where there was the very same conserved transcription factor binding site (TFBS) variation pattern than what we inferred from the expression levels. This means that in these examples, a TFBS variation could be associated to the higher and lower-level expression. Of course, in all these cases the heterozygous genotype resulted in an intermediate level of expression.
This experiment not only proves our hypothesis that the expression level of the genes on the autosomal chromosomes might follow an intermediate inheritance rule, but it also provides a technique for assigning transcription factor binding sites to genes and thus depicting the effect of the regulatory variations for the gene expression level. In the future work, we plan to develop an algorithm, which can automatically detect these cases from such kind of experiments.

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**The collaboration of transcription factors that determine macrophages**

Gergely Nagy

Macrophages do not only participate in tissue sampling for the immune system but – depending on their environment – are also capable of performing several other tasks. Their morphological and functional plasticity is basically allowed by few tens of transcription factors (TFs), some of which can be found in all macrophages, and the others provide tissue-specific cell functions. The major model of our research group is mouse bone marrow-derived macrophage, from which the sum of the genomic binding sites (cistrome) of nearly 40 TFs is known. Half of the TFs examined so far have tens of thousands of binding sites, and not only the PU.1/IRF8, RUNX1, C/EBP, and AP-1 TFs that are well known from macrophages, but also other ETS and leucine zipper (bZIP) proteins have large cistromes, however, their significance in macrophages is little known.
Our goal is to find a connection between the genomic binding sites occupied by different TFs and the gene expression of macrophages. Since 12 cistromes are available from the bZIP families, we started our analyses with the binding sites of these TFs. The cistromes of AP-1 (FOS/JUN) heterodimers showed the enrichment of TPA response elements (TREs), while those of other JUN, ATF, and CREB dimers included cAMP response elements (CREs). This is in line with decades of experience, but we also found unexpected motifs, e.g., in the cistrome of NFE2L2. NFE2 and BACH proteins form heterodimers mainly with small MAFs and, by binding MAF response elements (MAREs), have an opposite (activating or inhibiting, respectively) effect on gene expression. Our results suggest that NFE2L2 is not only able to form heterodimers with MAF proteins but also binds CREs with AP-1/CREB/ATF partners at active promoters, so it may play significant roles in gene regulation.

## ChIP-seq based determination and comparison of the human and mouse consensus transcription factor binding site sets

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Transcription factor based ChIP-seq experiments can show the possible binding sites throughout the whole genome. Of course, a ChIP-seq peak region does not necessarily mean the presence of the cognate binding site of the given transcription factor. Therefore, we have developed a pipeline, which takes the ChIP-seq experiments that use the same antibody and the related consensus binding sites to determine the consensus, ChIP-seq verified binding site sets in the human genome. Based on this analysis, we had also determined the peak summit positions, which give valuable information about the spatial location of the different transcription factors above the same binding site.
To facilitate the using of this plethora of information for other researchers, we have developed the ChIPSummitDB database (1). This database contains not only the summary of the peak summit regions at the different consensus binding site sets in an interactive form, but also a genome browser, which allows comparing the different consensus binding site sets with the peak regions from the different experiments.
The human ChIPSummitDB is based on 3727 ChIP-seq experiments and it contains 272 transcription factor consensus BS sets. To step further, we have started to process also the available mouse ChIP-seq data. In the first run, we have carefully chosen 9559 mouse ChIP-seq experiments. However, processing such a big amount of data needs special (bio)informatic arrangements. We had to re-design many parts of our pipeline and also had to allocate enough computing power, memory and storage. After the determination of the peak regions for each experiment and the mouse consensus TFBS sets, we used the human-mouse whole genome alignment-based chain files to transform the mouse genome coordinates into the human reference genome.

1. Czipa E, Schiller M, Nagy T, Kontra L, Steiner L, Koller J, **Pálné-Szén O,** Barta E, ***ChIPSummitDB: a ChIP-seq-based database of human transcription factor binding sites and the topological arrangements of the proteins bound to them***, Database, Volume 2020, 2020, baz141, https://doi.org/10.1093/database/baz141

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## Examination of antimicrobial and immunomodulatory peptides in Alzheimer's disease using network analysis of proteomics datasets and the DEAMP Database

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The reanalysis of genomics and proteomics datasets by bioinformatics approaches is an appealing way to examine large amount of reliable data. This can be especially true in cases such as Alzheimer's disease (AD), where the access to biological samples, along with well-defined patient information can be challenging. Considering the inflammatory part of AD, our aim was to examine the presence of antimicrobial and immunomodulatory peptides (AMPs) in human proteomic datasets deposited in ProteomeXchange and PubMed.
The DEAMP Database, containing all known human AMPs was constructed and used along with the datasets containing high quality proteomics data originating from the examination of AD and control groups. A throughout network analysis was carried out and the enriched GO functions were examined.
Less than 1% of all identified proteins in the brain were AMPs but all the alterations characteristic to AD could be recapitulated with their analysis. Our data emphasize the key role of the innate immune system and blood clotting in the development of AD. The central role of AMPs suggests their utilization as potential targets for mechanistic studies and future therapies.

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# Poster Session

### Thiamine Enhances Thermogenic Activation in Simpson-Golabi-Behmel Syndrome (SGBS) Adipocytes

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Targeting brown adipose tissue (BAT) activity has a therapeutic potential in treating obesity and metabolic syndrome by increasing energy expenditure. We have shown that thiamine (vitamin B1) increases the thermogenic activation in human primary subcutaneous and deep neck adipocytes. We aimed to investigate whether thiamine influenced the thermogenesis of Simpson-Golabi-Behmel syndrome (SGBS) adipocytes. This human preadipocyte cell line models the abdominal subcutaneous adipogenic precursors and has been successfully used in several studies investigating white and beige adipogenic differentiation.
We added excess amount of thiamine for 10 hours to the cell culture media of differentiated SGBS adipocytes. Thiamine increased the cAMP-stimulated upregulation of thiamine transporter 2 (ThTr2) expression in beige SGBS adipocytes at mRNA and protein level. The uncoupling protein-1 (UCP-1) mRNA and protein expression in both white and beige SGBS adipocytes was also elevated by thiamine. In addition, the mRNA expression of mitochondrial creatine kinase 2 (CKMT2), which can mediate UCP-1 independent thermogenesis, was increased by thiamine in white and beige SGBS adipocytes.
Our result confirmed that the increase of thermogenic activity induced by thiamine is not restricted to primary neck adipocytes, which are found in a limited amount in the human body, but could influence the heat production by SGBS adipocytes as well.

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**Study of biotinylated Zbtb46 transcription factor in mesodermal and myeloid cell differentiation using doxycycline-inducible murine pluripotent stem cells.**

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The transcription factor Zbtb46 is hallmark of dendritic cells, however, the exact function of this protein is still poorly characterized, especially outside the dendritic cell compartment. We have recently observed that, forced expression of this protein elicited a broad repressive effect at the early stage of ESC differentiation. To tackle this, our research group has created chemically (doxycycline) inducible Zbtb46 expressing transgenic murine pluripotent embryonic stem (ES) cell clones. To further investigate the effects of this factor we also generated biotin-tagged Zbtb46 expressing ES cell clones (the in vivo biotinylated peptide was inserted to the N terminal part of the Zbtb46 protein). The goal of this study is to test the impact of this biotin-tagged Zbtb46 (bio-Zbtb46) protein on the ES cell-based mesodermal and myeloid differentiation.
First, we have compared the Zbtb46 protein levels with intracellular flow cytometry testing bio-Zbtb46 and native Zbtb46 expressing transgenic ES cell clones. Our results showed elevated protein expression of bio-Zbtb46 in the transgenic ES cell lines upon doxycycline treatment. However, in case of undifferentiated ES cells the natural version of Zbtb46 protein showed a higher expression comparing the bio-Zbtb46 containing cells. On the other hand, the protein expression was similar of the bio- and the native Zbtb46 protein containing cells during differentiation. To characterize the effect of the modified Zbtb46 protein, the transgenic ES cells were differentiated with or without doxycycline to myeloid progenitors. On the 5th day of differentiation, the percentages of the Flk-1 positive cells decreased upon doxycycline induction suggesting that the mesoderm development can be inhibited with bio-Zbtb46. Similarly, decreased percentage of CD11b/CD45 positive myeloid cells were detected in the presence of bio-Zbtb46. Together, these results suggest that the bio-Zbtb46 can interfere with the mesodermal and myeloid development in ES cell derived progenitors. Interestingly, in case of the forced expression of the bio-Zbtb46 the inhibitory effect was less strong comparing the native Zbtb46. This result imply that the N-terminal modification of the Zbtb46 protein can influence the repressive activity of this transcription factor.
In conclusion, we successfully generated bio-Zbtb46 protein in our inducible transgenic ES-cell lines. Compared with the non-modified transgenic Zbtb46 phenotype, bio-Zbtb46 induced similar phenotypic characteristics in our doxycycline-inducible transgenic ES cells, although the repressive effect was weaker in the presence of the biotin-tag. In the future these bio-Zbtb46 expressing clones can be used for affinity purification of protein complexes associated with this transcription factor or to employ ChIP-seq experiments.
Funding: NKFIH K124890 and GINOP-2.3.2-15-2016-00044 PHARMPROT

**Differential expression of Nod-like receptors (NLRs) in the skeletal muscle of myostatin mutant Compact and wild-type Congenic mouse.**

Bodrogi Boglárka, Bíró Eduárd, Benkő Szilvia

Myostatin belongs to a transforming growth factor-β superfamily. It is mainly produced in muscle as a myokine and suppresses the growth of skeletal muscle by regulating the proliferation and differentiation of myoblasts. However, inhibition or loss of function mutation of myostatin leads to muscle hypertrophy and results in the development of so called *Compact* animals. The hypermuscular *Compact* mice carry a 12-bp deletion in the of the Myostatin (Mstn) propeptid sequence. Despite of the increased muscle mass, *Compact (Cmpt)* mice are characterized by decreased specific force generation. Ultrastructure of Cmpt skeletal muscle (like nuclei and mitochondria) shows several alterations compared to that of the wild type mice that leads to the generation of stressors and damaged molecules.

Nod-like receptors (NLRs) are intracellular patters recognition receptors that able to sense specific patterns of microorganisms or potentially harmful and dangerous molecules. NLRs play important roles in a wide range of biological processes: cytokine production, signal pathways, modulation of inflammation, embryo development, cell proliferation and cell death. Despite their diversity in different processes the expression and function of NLRs in skeletal muscle has hardly been investigated. We hypothesized that NLRs have an importan role in the muscle cells as well, and the severe structural changes of skeletal muscle in *Cmpt* mouse affect the expression and funciton of these NLRs. For this reason, we conducted a comparative analysis of NLR expression of *Tibialis anterior* muscle between *Cmpt* and *Congenic* wild-type mice strain using quantitative RT-PCR and Western blot methods.

We found significant changes in the expression of some NLRs that will be presented.

**Investigation the influence of agronomic management on the vineyard soil and trunk-bark microbiome of Vitis vinifera from Tokaj-Hegyalja and Pallag-Botanical and exhibitional garden**

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Several studies demonstrate that wine-terroirs are strongly influenced by the epiphytic microbiota of vines (*Vitis vinifera*) and environmental biodiversity representing a potential source of inoculum for wine fermentation. This study was carried out to explore the impact of biogeography and four different agronomical practices (conventional-1, -2, biodynamic and fellow) on microbial community composition and diversity. Next-generation sequencing technique was applied to thoroughly study the epiphytic bacterial community of vine bark and its relationships with soil microbiota. Two Hungarian geographical territories (Tokaj-Hegyalja and Pallag) were investigated. Tokaj-Hegyalja has been declared a World Heritage Site in 2002 under the name Tokaj Wine Region Historic Cultural Landscape which is the origin of „Tokaji aszú" wine, the world's oldest botrytized wine. In this study soil and vine bark samples were collected from vineyards evidencing different agronomic managements in

June, 2020. For each sample, the extracted genomic DNA samples were quantified and checked for purity. To sequence the V3 and V4 hypervariable regions of prokaryotic 16S rRNA genes, standard library preparation was performed according to Illumina 16S metagenomic sequencing library preparation protocol. In general, the alpha diversity metrices of soil samples proved to be significantly higher than those of the trunk-bark samples. As agronomic management regards, significantly lower biodiversity was experienced due to conventional agronomical practice in contrast to biodynamic and fellow irrespectively of the geography (Tokaj-Hegyalja vs, Pallag Botanical and Exhibition Garden). We managed to determine the core microbiota of wine-bark and soil samples of different vines and we estimated the beta-diversities of the different vitivinicultural terroirs. Our knowledge about the influence of geography and agronomical practices of vine bark- and soil associated microbiomes might also support the wine industry to understand the roles of microorganisms on the entire winemaking process, from vineyard to cellar.

**Phytonutrient-supplementation improved the health of the intestinal microbiota of *Cyprinus carpio***

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As the human population is expanding, there is an immense rising need for the aquaculture sector to overcome main efficiency problems, and to use resources in a greener way. It is a promising approach to improve the health of the animals by modulating the gastrointestinal tract microbiota. A feeding trial was carried out to investigate the phytonutrient-induced changes in the gastrointestinal tract microbiota of common carp (*Cyprinus carpio*). A basal diet (BD) was applied as a negative control (did not use any supplement), and the following dietary supplements were used at a 1% dose: anthocyanin (ANTH) enriched diet from the production of Hungarian sour cherry, synbiotics (SYN) from fermented corn, fermentable oligosaccharides (fOS) from Hungarian sweet red pepper seeds, and carotenoids (CAR) from Hungarian sweet red pepper pulps. These components are typical by-products in the food industry. Through the four time points of the experiment, the gut contents of the animals were collected. Microbial DNA samples were isolated, and V3-V4 16S rRNA gene-based metagenomic sequencing was performed. According to our data, there is no significant differences in the growth performance of differently fed common carp juveniles. As a result, phytonutrients improved the community diversity of GIT microbiota, increased the abundances of beneficial Clostridium and Lactobacillus; and decrease the abundances of potentially pathogenic bacteria: Shewanella, Pseudomonas, Acinetobacter and Aeromonas. Through the experiment, the phyla Proteobacteria, Tenericutes and Chlamydiae showed a positive correlation with the body weight, whilst Spirochaetes and Firmicutes exhibited negative correlations. We hypothesize that the application of phytonutrients as supplement in aquaculture routine might be a reasonable green approach for improving animal health and easing the usage of antibiotics.

## Circulating miRNA profiling in plasma samples of glioblastoma patients

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Introduction: Glioblastoma (GBM) is the most common primary malignant brain tumor in adults. Histological identification of GBM requires tumor tissue however morbidity of surgical resection can be as high as 10-30%. Therefore there is a growing interest for non-invasive liquid biopsy aiming early GBM diagnosis as well. Detection of deregulated miRNAs in bio-fluids from GBM patients suggests that the altered pattern of miRNAs have the potential to become a non-invasive biomarker.
The aim of our study was to compare miRNA profiles of plasma samples derived from GBM and control patients.
Materials and methods: MiRNA expression analysis was performed from plasma samples of 6 glioblastoma patients and of 6 control individuals applying the nCounter Analysis system (NanoString Technologies) and the nCounter Human v3 miRNA Panel with 798 unique miRNA barcodes on it. Analysis of raw miRNA data was performed using the web based miRNet, miRTarBase, and TargetScan software programs. 10 "housekeeping" miRNA counts were used for the normalization.
Results: Analysing miRNA expression profiles 59 miRNAs showed significantly different expression between controls and patients. From these only one miRNA was downregulated while 58 miRNAs were upregulated in the plasma sample of GBM patients. Validation of these results by determining the expression level of hsa-miR-433-3p, hsa-miR-29a-3p, hsa-miR-195-5p, hsa-miR-362-3p, hsa-miR-331-3p, hsa-miR-29a-3p via quantitative real-time PCR analysis using the miScript SYBR Green PCR Kit (Qiagen) is in progress. Expression levels of the selected miRNAs are calculated using comparative cycle threshold (Ct) method, and miR-16 was selected as internal control.
Conclusion: Our study with the nCounter Analysis system identified significantly elevated expression of circulating miRNAs in plasma samples of GBM patients that needs further validation.

## Ikzf1 and Runx3 transcription factors negatively modulate the mesodermal development from pluripotent embryonic stem cells

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To investigate the potential of embryonic stem (ES) cells for patient-centered approach therapy in cancer and malignant diseases, we need to know in detail the process in which direct the stem cells to differentiate. To accelerate the ex vivo differentiation of ES cells we probed the effects of various transcription factors using chemically (doxycycline) inducible mouse embryonic stem cell lines. In our laboratory we have previously found that forced expression of Runx3 or Ikzf1 positively regulated the myeloid dendritic cell development and

maturation. In this study we analyzed the impact of these factors during the early stage of ES cell differentiation, especially the mesoderm cell formation was assessed. To monitor the effects of Ikzf1 or Runx3, first the transgenic ES cells were expanded and selected by G418 for three days. After that, cells were differentiated by co-culture with a layer of OP9 cells for five days with or without transgene induction. To evaluate the mesoderm cell formation we determined the Flk-1 and Pdgfr-alpha expression with flow cytometry, in addition, the transgene expressions were also measured with quantitative real-time PCR.
Unexpectedly, our experiments revealed that the mesoderm differentiation was strongly inhibited in the presence of the transgenic Ikzf1 or Runx3 transcription factors. Interestingly, previously we have observed a similar repressive effects upon the forced expression of an additional transcription factor (Zbtb46). There results raise the issue that forced expression of any transgenes can repress the mesoderm cell formation in this ex vivo differentiation model. It is also possible that the doxycycline treatment itself can elicit this inhibitory effect. To exclude these possibilities, additional ES cell lines were analyzed. Importantly, we failed to detect any inhibitory effects when the non-transgenic ES cells (E14 cells) were treated with doxycycline. Similarly, the mesoderm differentiation was normal upon the induction of report transgenes (EGFP, or mCherry). These findings suggest that the investigated transcription factors can selectively inhibit the mesoderm commitment. We plan to carry out more experiments testing additional transcription factors. In addition, we intend to characterize molecular mechanism of the transcription factor dependent mesoderm lineage suppression.
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**Examination of zymosan induced transcriptomic changes of Egr2 transcription factor deficient alveolar macrophages**

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Alveolar macrophages (AMs) as a part of innate immunity take part in the primer protection against inhaled pathogens. High number of studies proved that near adaptive immunity the innate immune system also able to generate immune training which leads more efficient action during inflammation. The examination and understanding of innate immune training mechanisms in AM are especially important from therapeutic point of view.
Early Growth Response 2 (Egr2) is a transcription factor which fulfills important role in the differentiational process of numerous cell types but its importance in epigenetic regulation of macrophage-mediated inflammatory response has not characterized yet. Moreover, based on our previous studies the Egr2 regulates the Dectin-1 receptor which is the major element in creation of trained innate immunity response of macrophages.
In our study, we applied Egr2 LysozymeM-Cre mice to examinate the transcriptomic alterations upon the loss of Egr2 in AMs after zymosan treatment which acts as a ligand of Dectin-1. We treated the mice intranasally using 300 μg zymosan bioparticle. After 6- and 24-hour incubation we isolated the AMs from lung using bronchoalveolar lavage. We performed RNA-sequencing to describe the changes in transcriptome.

Our results support that Egr2 deficiency highly influence the zymosan induced inflammatory response of AMs. We identified more than 2000 genes which show significant alterations between the wild-type and Egr2 KO mice during this process. We separated gene clusters showing different expressional dynamics and potential functional impair in pathogen elimination. Based on our prediction the Egr2 acts as a modulator of Transforming Growth Factor (TGF) β signaling pathway.
Our findings point out the potential transcriptional regulatory role of Egr2 in trained innate immune mechanisms and anti-fungal activity of alveolar macrophages.
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## ROLE OF NLRP3 INFLAMMASOMES IN THE ANTIMICROBIAL ACTIVITY OF HUMAN PLASMACYTOID DENDRITIC CELLS

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**Introduction:** Plasmacytoid dendritic cells (pDC) as professional type I interferon-producing cells are the major coordinators of antiviral immune responses. Their role in antibacterial immunity is less explored, although it is known that pDCs may play a protective role against secondary infections developing during the late immunosuppressive phase of sepsis. So far no data are available about the NLRP3 inflammasome activity of pDCs, which could be involved in their antimicrobial response. Thus our aim was to investigate the NLRP3-mediated antibacterial responses in human pDCs.
**Methods:** In our experiments, pDCs were exposed to various species of live bacteria, and then the expression of NLRP3 inflammasome components was examined at mRNA and protein levels using Q-PCR and western blot. Active IL-1β secretion of the cells was examined in the presence of specific NLRP3 activators and inhibitors by ELISA.
**Results:** Our results indicated that human pDCs are able to express the components of NLRP3 inflammasome, but the production of pro-IL-1β is highly dependent on the type of microbial stimulus. We observed that pathogenic bacteria have a higher capacity to induce NLRP3 activation, in contrast to commensal species. After pre-treatment with pathogenic bacteria, pDCs were also able to secrete the mature, cleaved form of IL-1β upon nigericin stimulation. The secretion of cleaved IL-1β was suppressed in the presence of a specific NLRP3 inhibitor indicating that the IL-1β secretion was NLRP3 dependent in pDCs.
**Conclusion:** Our results show that the NLRP3 pathway is functional in human pDCs, thus suggest that beside their strong antiviral properties, pDCs may also be involved in the NLRP3 inflammasome-dependent IL-1β-mediated inflammatory responses.
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**High-throughput RNA sequencing revealed invasive aspergillosis specific (IA induced) miRNA signatures in the whole blood samples of onco-hematology patients**

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Aspergillosis may occur as a serious complication of hematological malignancy. Delay in antifungal therapy can lead to an invasive disease resulting high mortality in onco-hematology patients. Microbiological laboratories still struggle to achieve timely and adequate diagnosis in the case of fungal infections. MiRNAs are a class of typically small non-coding RNAs which can regulate gene expression post-transcriptionally by miRNA-mRNA interactions. During the past decade the connection between extracellular miRNA levels and several pathological processes, including different infections are increasingly recognized. The prime aim of this study was to identify aspergillosis specific circulating miRNA profiles in onco-hematological patients. Altogether 20 whole blood specimens were gathered, and their miRNA transcriptome was deep-sequenced on Illumina NextSeq 500 platform. Differential expression analyses revealed microRNAs which were significantly altered in their expression due to aspergillosis.

**Study the role of miR30 family members in ovarian cancer**

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Introduction: Ovarian cancer is the 5th most common cause of cancer death among women. The application of estrogen based hormone replacement therapy highly increases the risk for ovarian cancer. MiRNAs are involved in the development of cancer as oncomirs or tumor supressors. MiRNAs are also present in extracellular fluids (cell-free miRNAs) that are considered to be promising biomarker candidates in non-invasive diagnostics. The aim of our study was to investigate the significance of miR30 family members in ovarian cancer that are considered to be tumor supressors and involved in the regulation of cell cycle, apoptosis and invasiveness of cancer.

Materials and methods: We compared the cell-free expression of miR30a-3p, miR30a-5p, miR30d-5p and miR30e-5p in the plasma samples of patients with ovarian carcinoma (n=21) and healthy controls (n=37) by qPCR. We also quantified the expression of miR30s in human epithelial ovarian cell cultures (PEO1: estrogen sensitive cell line) in response to estradiol treatment. Finally we studied the effect of estradiol to cell viability by MTT test.

Results: According to our results miR30a-3p and miR30e were significantly overrepresented in the plasma samples of patients with ovarian carcinoma ($p<0.05$) compared to the healthy controls. MiR30s had high basal expression in the cell lysates of PEO1 cultures. Their cell-free expression was also detectable in the supernatant of PEO1 cultures. The expression of miR30a-5p, miR30d-5p and miR30e-5p was highly up-regulated in response to high estradiol doses (50, 100 μM) that was not observed in the case of miR30a-3p. The expression of

miR30a-3p was down-regulated in response to 10 nM estradiol exposure, that concentration proved to have proliferative effect previously. High estradiol doses significantly decreased cell viability. Target analysis of miR30s revealed that miR30a-5p, miR30d and miR30e share several targets involved in apoptosis. Mir30a-3p targets several genes involved in cell proliferation.
Conclusions: According to our results miR30a-5p and miR30e might be promising biomarker candidates in the non-invasive diagnostics of ovarian cancer. Furthermore, mir30a-3p might be involved in the proliferative effect of estradiol. However, miR30a-5p, miR30d and miR30e might mediate the stress-response induced by high estradiol doses.
Funding: The research was supported by the EFOP-3.6.3-VEKOP-16-2017-00009 project co-financed by EU and the European Social Fund.

**Development of protease sensitive mouse pancreatic lipase**

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Mutations in pancreatic secretory proteins may predispose to chronic pancreatitis, an inflammatory disease of the human pancreas, via two independent pathological pathways: increased intrapancreatic trypsinogen activation, and protein misfolding. Recently, our laboratory in a large collaborative study has identified a few rare heterozygous pancreatic triglyceride lipase (PNLIP) mutations in German and French chronic pancreatitis cohorts. Genetic analysis in combination with functional biochemical experiments indicated that five PNLIP mutations (P245A, I265R, F300L, S304F, F314L) that make the enzyme protease sensitive are highly enriched in pancreatitis, and the disease mechanism seemed to be independent of the known pathways.

Our aim was to study the effect of human PNLIP mutations on the proteolytic stability of the mouse orthologue enzyme.

The coding sequence of Pnlip was cloned into the mammalian expression vector pcDNA3.1(-) using pancreatic cDNA of mouse strain FVB. In addition, a 10-histidine affinity tag was engineered onto the C terminus of Pnlip. Mutations were introduced with overlap extension PCR mutagenesis and were verified with capillary sequencing. HEK 293T cells were transiently transfected with the plasmid DNA. Secretion of Pnlip into the conditioned medium was followed by lipase activity measurements, and SDS-PAGE and Coomassie staining. Pnlip in cell lysates was identified with SDS-PAGE and western blotting using HRP-conjugated penta-His antibody. Pnlip was purified from conditioned media using Ni-affinity chromatography. Proteolytic degradation of Pnlip in the presence of trypsin was followed with SDS-PAGE and Coomassie staining.

Pnlip wild type and variants I265R, F300L, S304F and F314L were readily secreted into the media by transfected cells. In contrast to its human counterpart, the mouse P245A variant was detected only in cell lysates without secretion suggesting normal translation followed by protein misfolding and intracellular retention. Pnlip wild type was resistant to proteolytic cleavages by the pancreatic digestive protease, trypsin over the one-hour-long incubation. Trypsin degraded Pnlip variant I265R quickly, while variants F300L, S304F and F314L were cleaved with a notably slower rate. In agreement with the human variants, the digestion of the I265R Pnlip generated multiple relatively stable protein bands migrating at 15-30 kDa range on the protein gel.

Our observations demonstrate that protease sensitive phenotype of human PNLIP variants can be reproduced with the mouse enzyme. The results will contribute to the development of a knock-in pancreatitis mouse model.

**Biochemical characterization of SARS-CoV-2 $M^{pro}$**

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Coronavirus Disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). >71 millions of people have been infected and >1.6 millions of COVID-19-related deaths were reported to date (2020.12.12). The worldwide coronavirus pandemy became one of the greatest challenges of the 21$^{st}$ century. Effective therapies are highly desirable, one of the promising drug target is the SARS-CoV-2 main protease ($M^{pro}$) due to it crucial role in the viral replication cycle, therefore, characterisation of this viral enzyme may provide valuable information.

Biochemical characterization of related SARS-CoV and MERS-CoV proteases are known from the literature, while the information about the enzymatic characteristics of SARS-CoV-2 $M^{pro}$ are still limited. Therefore, we aimed to investigate the effects of different conditions (pH, temperature, ionic strength, and organic solvents) on enzyme activity to set up an optimized environment for subsequent inhibitory studies.

For enzymatic reactions, we expressed, purified and applied a $His_6$-MBP-TSAVLQSGFRKM-mEYFP recombinant fluorescent protein substrate which represent one of the naturally occurring cleavage sites of the SARS-CoV-2 $M^{pro}$. To detect product formation, cleavage of the recombinant substrate was followed by SDS-PAGE and by a fluorimetric assay.

We found that the SARS-CoV-2 $M^{pro}$ shows relatively higher activity at lower ionic strength (<100 mM NaCl), and highest activity was detected close to neutral pH (pH 6.8) and the temperature optimum was also close to 37 °C. The investigated enzymatic characteristics of SARS-CoV-2 $M^{pro}$ were compared to those of SARS-CoV $M^{pro}$ known from literature. Furthermore, we found that different organic solvents (applied in 1 v/v% final concentration) have no effect on the enzymatic activity and do not interfere with the applied magnetic affinity bead-based fluorimetric method.

Our results provide valuable information about the enzymatic characteristics of SARS-CoV-2 $M^{pro}$, and the optimized conditions may be applied in enzymatic reactions for the screening of different potential inhibitor molecules.

**Long-Term Thiamine Treatment Elevates Thermogenic Competency of Human Primary Neck and SGBS adipocytes**

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Brown adipose tissue (BAT) dissipates energy as heat via the mitochondrial uncoupling protein-1 (UCP-1) activity. The activation of BAT was postulated as a potential therapeutic target to treat obesity. Therefore, novel agents that can stimulate the differentiation and recruitment of brown or beige thermogenic adipocytes in humans are important subjects for investigation. Our preliminary data showed that short-term thiamine (vitamin B1) treatment increased the thermogenic activation of human subcutaneous (SC) and deep neck (DN) adipocytes. Then we aimed to investigate the effect of continuous thiamine treatment during adipocyte differentiation on the expression of thermogenic marker genes and proteins.
We treated primary human adipogenic progenitors that were cultivated from SC and DN adipose tissue and SGBS preadipocytes with excess amount of thiamine during their 14 days long differentiation program. Long-term thiamine administration did not influence the mRNA and protein expression of either thiamine transporter 1 (ThTr1) or 2 (ThTr2) in human neck and SGBS adipocytes. The mRNA and protein expression of UCP-1 was increased in white-differentiated SC neck and SGBS adipocytes upon long-term thiamine treatment. Our preliminary data also showed there was an increasing trend in the expression of mitochondrial complexes II, III, and IV in white-differentiated SC adipocytes. In addition, long-term thiamine supplementation led to the elevation of CKMT2, which can mediate UCP-1 independent thermogenesis, and the CIDEA thermogenic marker at mRNA level, in SC and DN adipocytes.
Our results present a novel approach to stimulate human adipocyte browning *ex vivo* by providing excess amount of thiamine into the differentiation media. Further experiments are required to characterize the morphological and functional properties of thiamine treated adipocytes.

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# Section 6

### Arsenic trioxide (ATO) treatment together with all-trans retionic acid (ATRA) attenuates the cell survival potential of acute promyelocytic cells transglutaminase 2 (TG2)-dependent manner

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All-trans-retinoic acid (ATRA) therapy is one of the most frequently used to treat acute promyelocyte leukaemia (APL). Administration of ATRA induces terminal differentiation of leukaemic cells towards neutrophil granulocytes, while arsenic-trioxide (ATO) has recently been identified as another effective drug for apoptosis induction and for the elimination of the LIC (Leukaemia iniciating cells). ATO, in combination with ATRA, shows a synergistic effect, which further prolongs the survival of APL patients in a dose-dependent manner. ATO also affects different transcription factors resulting in activation of the cellular signalling pathways leading to, among others, an enhanced reactive oxygen species (ROS) generation by the NADPH-oxidase system. These events explain the ability of ATO to induce partial differentiation and apoptosis, leading the remission in relapsed APL patients with the initiation of the degradation of the PML-RARα. This differentiation method results in up and

down-regulation of several hundreds or thousand genes to generate functional neutrophil granulocytes. One of the most up-regulated genes in ATRA induced differentiation of NB4 cells is the tissue transglutaminase (*TG2*). Silencing/knocking out of TG2 expression in NB4 cells revealed that TG2 is required for adhesion, migratory, the phagocytic capacity of neutrophils, superoxide (ROS) production and inflammatory cytokine/chemokine production [*Balajthy et al., Blood 2006, Jambrovics et al., Heamatology 2018, Jambrovics et al.,Cancer 2020*].

To investigate the role of TG2 in the cell survival processes, NB4 cells were treated with ATRA + ATO in two different combinations, where we found that without the TG2, NB4 cells were more senzitive to the arsenic induced apoptosis. This phenomenon supports our hypothesis about TG2 protective role via TG2-AKT-mTOR signaling pathways.

**An attempt to reveal the biological function of human transglutaminase 4 in the human saliva**

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Transglutaminase 4 (TG4) is one of the less-studied member of the transglutaminase enzyme family. It was thought that TG4 has a prostate-specific expression, but it is also present in vascular smooth muscle cells, in the vesicular fraction of the urine, and secreted into the saliva. We aimed to reveal the biological function and significance of TG4 in the human saliva.
A monoclonal antibody detects TG4 with around 55 kDa size in the human saliva using denaturing SDS-PAGE. MS analysis was used to confirm the presence of TG4 in the saliva and identify the site of its potential proteolytic cleavage. Still, any TG4 tryptic peptide was not identified, probably due to the high polymorphism, extensive post-translational modifications, and low level of TG4. For enrichment of the protein from human saliva, antibodies were tested, but biolayer interferometry experiments demonstrated a low affinity of the available monoclonal antibodies towards native TG4.
TG4 is present in the saliva's insoluble fraction, and as TG4 was detected in the vesicular fraction of the urine, we isolated extracellular vesicles from the saliva. TG4 was successfully enriched in the vesicles, and also the full-length enzyme could be detected in this fraction. The extracellular vesicle samples were also analysed by mass spectrometry. However, TG4 was not detected, but several extracellular vesicle specific proteins were confirmed in the samples. The most enriched ‘Cellular Components’ based on STRING analysis of the identified proteins were ‘secretory granule lumen’, ‘secretory granule’ and ‘extracellular exosome’. The most enriched ‘Biological Processes’ were various immune functions and ‘vesicle-mediated transport’.
Application of a site-specifically biotinylated TG4 resulted in the identification of 292 potential interaction partners. They overlap with extracellular vesicle enriched proteins from saliva. The highest hits for ‘Biological Functions’ among the interaction partners were related to ‘calcium-dependent phospholipid binding’, ‘enzyme inhibitor activity’, and ‘lipid binding’.

In order to develop a cellular model for studying TG4 expression and secretion, 293AD – a transglutaminase-free epithelial cell line – was transfected with a TG4 cDNA containing vector. 293AD cells expressed TG4 in full-length form, and further experiments are in progress.
In the future, co-immune precipitation and Blitz measurements are planned to confirm the interaction between TG4 and its potential, identified salivary interacting partners.

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**Serum reaction of celiac disease patients to gliadin peptides p31-43 and p57-68 arise from deamidation independent cross-reactive γ-gliadin specific antibodies**

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Celiac disease (CeD) is a chronic, systemic immune disorder with autoimmune features where ingestion of gluten induces antibodies against gliadin peptides and a self antigen, transglutaminase 2 (TG2). TG2 is contributing to the pathomechanism by binding and deamidating gliadin peptides.
Wheat gluten prolamin peptides, α-gliadins play key role in innate immune activation and disease associated DQ2 restricted T-cell stimulation, however specific B-cells reaction targeting these peptides was not reported.
We investigated two highly immunogenic α-gliadin peptide fragments (p31-43 and p57-68) and a well known B-cell stimulatory γ-gliadin peptide (γ-Glia). First we examined gliadin peptide deamidation by human TG2 enzyme and based on the generated sequences we selected 8 recombinant gliadin peptides. Applying biolayer interferometry we quantified serum reactivity of CeD patients toward the different native and deamidated α- and γ-gliadin peptides. Further, in a series of risk children longitudinally followed since birth, we investigated the appearance and specificity of early age gliadin specific antibodies. CeD patient derived gliadin specific antibodies affinity purified with each recombinant peptide were tested for their cross-reaction with the peptides.
We identified unreported deamidation sites in case of p31-43 gliadin peptide and detected significant serum reactivity toward the p31-43 and p57-68 peptides. Gliadin specific antibodies predominantly recognize γ-gliadin peptides and do not display deamidation dependent recognition. The α-gliadin reactive antibodies appear at later age and at a lower extent compared to the γ-gliadin reaction. Based on the isolated CeD patient derived antibody populations we can conclude that α-gliadin reactivity arise from cross-reacting γ-gliadin specific antibodies, as p31-43 and p57-68 derived antibodies recognize the γ-gliadins much more efficiently.

These finding reveal important aspects of the humoral anti-gliadin immunity that contributes to our understanding of CeD pathomechanism.
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**Misfolding of pancreatic lipase (PNLIP) mutants leads to endoplasmic reticulum stress and pancreatitis**

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Chronic pancreatitis, a severe inflammatory disease of the pancreas, commonly occurs as a result of genetic predisposition in children. Misfolding of mutant proteins and consequent endoplasmic reticulum (ER) stress in the human pancreas regularly lead to the development of the disease. Recently, in two European pancreatitis cohorts of young patients, rare pancreatic triglyceride lipase (PNLIP) mutations (A174P, C254R, G233E, V454F) causing reduced PNLIP secretion were identified.
Our aim was to determine whether these mutations are pathogenic or harmless variants.
PNLIP wild type and variants were introduced into HEK 293T and AR42J acinar cells by plasmid transfection and adenoviral transduction, respectively. Lipase secretion was studied with activity measurements and SDS-PAGE followed by Coomassie staining or western blotting. The levels of ER stress markers XBP1 mRNA splicing and BiP expression were determined with PCR-based techniques.
In contrast to the wild-type PNLIP, which was readily secreted into the medium, mutations A174P, C254R, G233E, V454F caused severe secretion defect of the enzyme. Western blot experiments verified that PNLIP mutants are expressed and accumulated intracellularly as insoluble protein aggregates. Consistent with our previous findings, expression of the PNLIP mutants resulted in ER stress as shown by significantly increased XBP1 mRNA splicing and elevated level of the ER chaperon BiP.
These observations demonstrate that PNLIP mutations triggering misfolding and secretion defect of the enzyme are risk factors of chronic pancreatitis.

**Development of a bio-layer interferometry-based protease assay**

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Proteases belong to the most intensively studied enzymes, due to their importance in numerous physiological processes of various biological systems, in molecular biological applications, and due to the clinical correlations of protease-related deficiencies. Therefore, the methods that are suitable for the investigation of proteases by a cost-efficient, reliable and sensitive real-time monitoring assay are highly desirable.

In this study we designed a protease assay method, using HIV-1 protease as a model enzyme. This novel *in vitro* approach is based on the use of $His_6$-MBP-mEYFP recombinant fluorescent protein substrates - that were designed previously by our research group - and the measurement of enzyme activity is carried out by bio-layer interferometry. The processing of the substrates attached to the sensor is followed by continously detecting the change of the

layer's thinkcness over the time. The proteolysis can be detected by this method within seconds after the initiation of the reaction.

The initial rate of the signal's decrease was found to show linear correlation with the enzyme concentration, thus, can be used for comparative analysis of different substrates. We compared cleavage efficiencies of different substrates, including such substrates that may be applicable to study the extended substrate binding sites of HIV-1 protease. The results of bio-layer interferometric assay were in agreement with those of enzyme kinetic measurements. We proved that the method may be suitable to study the effects of protease inhibitors on enzyme activity, as well. Finally, we showed that not only purified substrates but total cell lysates containing the fluorescent substrates may also be used for the measurements, making the measurements more time- and cost-efficient.

In this work we developed a novel approach for protease activity measurements, however, bio-layer interferometry is used mainly to study protein-protein interactions. The developed protease assay may be suitable for highly sensitive screening of multiple substrates and inhibitors in a small-volume.

# Section 7

**Analysis of changes in the cellular proteome and transcriptome following HIV infection and the role of HIV-2 Vpx.**

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The human immunodeficiency viruses (HIV-1 and HIV-2) share a similar genomic and organizational structure. These viruses rely on host cell machinery for replication, and compared to HIV-1, responses of the host cell to HIV-2 infection is less well studied.

One of our aims was to characterize the remodeling of the cellular proteome and transcriptome in early time-points (0-26 hours), following transduction of HEK-293T cells by HIV-1 and HIV-2 pseudovirions, using mass spectrometry and transcriptomic analysis.

Vpx is an accessory protein that is present only in HIV-2 and its predecessor, the simian immunodeficiency virus of sooty mangabeys (SIV/smm). Its function has not yet been thoroughly characterized. In our previous studies, HIV-2 Vpx was responsible for dampening the infectivity of HIV-1 in dual transduction assays. We therefore set out to elucidate the function of this accessory protein, and reveal its cellular interaction partners, as well as the transcriptomic changes induced upon transfection of the cells with this protein, using pull-down immunoprecipitation, and transcriptomic analysis.

Our results indicate that in the first 2 hours of transduction, 7 proteins were significantly down-regulated by HIV-2, and 5 by HIV-1. Among these proteins were the Non-POU domain-containing octamer-binding protein (NONO), heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) and Serine and arginine rich splicing factor 6 (SRSF6), all of which have been shown to augment viral replication in the late-phase of infection. Interestingly, chaperonin containing TCP1 subunit 8 (CCT8) was significantly down-regulated only in the case of HIV-2, overexpression of this protein was found to associate with progression towards AIDS. Expression level of genes involved in mRNA splicing, transport, DNA repair and cytoskeletal reorganization was also differentially altered. Pull-down assay showed that wild-type Vpx interacted with many proteins involved in splicing,

packaging of pre-mRNA, nuclear export and translation. Our findings shed light on the role of HIV-2 Vpx, and highlight the differential changes in the cellular proteome and transcriptome in the early-phase of HIV-1 and -2 infection.

**Evaluation of serum sample processing methods for metabolomics and proteomics analyses**

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Type 2 diabetes (T2D) and obesity are most common and interrelated metabolic conditions. Recent studies have reported that serum amino acid and protein profiles alterations may be associated with the risk of T2D and obesity. In this study, our aim was to evaluate an appropriate sample processing method for metabolomics and proteomics analysis of serum samples collected from patients with T2D, patients with obesity, and a matched control group. Serum samples for metabolomic analysis were divided into three groups and processed with different methods, separately. The level of 23 amino acids and 9 biogenic amines was examined in the filtrate from centrifugal filtration (3kD Nanosep), supernatant from acetone precipitation (AP), and upper phase from methanol-chloroform extraction (MCE). The samples were derivatized with AccQ-Tag derivatization kit (Waters) and subsequently analyzed by H-Class UPLC system (Waters).
For the protein analysis, the protein fraction of MCE and AP was examined along with the untreated serum. In case of each sample type in-solution digestion, filter-aided sample preparation (FASP), and ZipTip (C18) digestion was carried out with trypsin. The tryptic digests were analyzed by Orbitrap Tribrid (Thermo Scientific) mass spectrometry system coupled with Easy-nLC UPLC (Thermo Scientific).
Concentration of all amino acids was significantly higher in the MCE sample compared to others. Of 7 biogenic amines detected, the concentration of 5 was significantly higher in the MCE sample. For protein analysis, the digestion method with the best results was FASP.
In conclusion, MCE with FASP method can be applicable for both metabolomics and proteomics analyses in our future projects.

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**Annotation Pipeline of Grape and Wine Metabolites detected by High-Resolution Mass Spectrometry.**

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(1) Background: Due to the advancements of analytical instrumentation platforms, a remarkable amount of data is produced in a matter of just a few measurements. High-resolution mass spectrometry enables the high-quality detection of hundreds of compounds from complex solutions of plant origin like wine. In this era of big data, access to rapidly increasing biological and chemical information makes possible to gain insight into the biological activities of the identified compounds published in the scientific literature. The size

and complexity of the datasets necessitate the use of application programming interfaces (APIs) of relevant chemical and publication databases.
(2) Objectives: To build a programmatic workflow to retrieve information on the biological and pharmacological effects of the individual compounds measured with liquid chromatography-mass spectrometry (LC-MS) in 7 "furmint" and 5 "aszú" Hungarian wine samples from Tokaj region.
(3) Results: ChemSpider, PubChem Compound and BioAssay, then PubMed database searches were carried out via their respective APIs. The LC-MS analysis yielded 288 molecules: 137 molecules were found to have biological activity in the PubChem BioAssay database, and 153 had scientific articles connected to them in PubMed; dozens of them were examined in functional studies, and reported to exert antiviral, anti-inflammatory and anticancer activities.
(4) Conclusions: Programmatic retrieval of published biological and chemical data of the detected grape and wine metabolites provided overall insight into their biological effects. Since nearly half of the molecules in the entire dataset had no recorded biological activity, in the future the integration of further databases (e.g. Web of Science, ChemAxon) is planned, and considering the ever-expanding databases, recurring searches of this and similar datasets are recommended.
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**Investigation of the catalytic cycle of ABCG2 in permeabilized cells**

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The development of multidrug resistance (MDR) is often responsible for the failure of cancer chemotherapy. MDR is frequently caused by the overexpression of ATP-binding cassette (ABC) transporters, such as ABCG2. ABCG2 is an active transporter that can export the majority of drugs applied in cancer treatment from tumor cells. Similarly to other ABC transporters ABCG2 is believed to alternate between nucleotide-free inward and nucleotide-bound outward facing conformations upon its catalytic cycle. Previous studies revealed that the reactivity of ABCG2 with the 5D3 anti-ABCG2 mAb is dependent on the conformational changes of the transporter.

We have studied the roles of the 5D3 distinguished conformers in the catalytic cycle of ABCG2. We have demonstrated that removal of nucleotides from cells permeabilized by bacterial toxins can synchronize ABCG2 molecules in a 5D3-reactive conformation. Conversely, addition of ATP at conditions either allowing (presence of ATP/$Mg^{2+}$ at 37 °C) or preventing ATP hydrolysis (absence of $Mg^{2+}$ or low temperature) switches ABCG2 into a 5D3-dim state in a nucleotide concentration dependent manner. Interestingly, ADP also brings about a switch of ABCG2 to the 5D3-dim conformation, although it binds to the transporter with lower affinity compared to ATP. Taken together, these results suggest that the conformational changes between the 5D3-reactive and non-reactive ABCG2 conformations

are caused by nucleotide binding and dissociation. Addition of vanadate ($V_i$) can trap ABCG2 molecules in a post-hydrolysis conformation with low 5D3 affinity. In our kinetic studies the ABCG2 substrate quercetin increased the rate of the formation of the vanadate trapped post-hydrolytic complex in accordance with the substrate stimulation of the ATPase activity of the protein.

To shed light on the details of the ABCG2 mediated drug translocation process we studied the substrate binding affinities of the different ABCG2 conformers. We have found that the inward facing conformation possesses higher substrate affinity compared to the outward facing conformation. We also proved that ATP binding is sufficient to decrease the substrate-affinity and induce substrate release, while the role of ATP hydrolysis is to switch the transporter back to the high substrate affinity inward facing conformation to initiate a new transport cycle.

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**The Effects of Sour Cherry Flavonoids and Anthocyanins on Pgp activity**

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P-glycoprotein (Pgp) is a member of one of the largest family of active transporter proteins called ABC transporters. Thanks to its expression in tissues with barrier functions and broad substrate spectrum it is an important determinant of the absorption, metabolism and excretion of many drugs. Pgp and/or some other drug transporting ABC proteins (e.g., ABCG2, MRP1) are overexpressed in nearly all cancers and cancer stem cells by which cancer cells become resistant against many drugs. Thus, Pgp inhibition might be a strategy to fight against drug-resistant cancer cells. Previous studies have shown that certain flavonoids and anthocyanins interact with human Pgp. We have tested the effect of 15 sour cherry origin compounds on the basal and substrate stimulated ATPase activity, calcein-AM and daunorubicin transport as well as the conformation of Pgp.

We have found that quercetin, narcissoside and ellagic acid inhibited the ATPase activity of Pgp and increased the calcein accumulation of Pgp-positive cells. Pgp-negative cells exhibited high calcein accumulation which was not affected by flavonoids. Transferulic-acid, oenin, myrtillin, caffeic acid did not affect the ATPase of Pgp and the intracellular accumulation of calcein suggesting that these compounds do not interact with Pgp. We did not find strong correlation between the results of ATPase assays and substrate accumulation tests. Although, quercetin-glucoside had inhibitory effect in ATPase assay it was ineffective in calcein assay probably because of its lower membrane permeability. Interestingly catechin and epicatechin behave differently, although they are stereoisomers.

We have found that the combination of quercetin and cyanidin-3O-sophroside synergistically increased the accumulation of calcein and daunorubicin into the Pgp-positive NIH 3T3 cells, since the combined effect of the two flavonoids was greater than the sum of the individual effects of each compound. Interestingly, when applied alone quercetin decreased, while cyanidin-3O-sophoroside weakly stimulated the ATPase activity of Pgp, however, when they were added together a strong inhibitory effect was experienced. The two compounds did not have significant effects on the UIC2 reactivity when they were applied separately. But, an almost three fold increase of UIC2 reactivity was detected when the two compounds were added simultaneously in accordance with their strong Pgp inhibitory effect. Taken together our data suggest complicated interactions between flavonoids and the complex

drug binding pocket of Pgp. Our results also call the attention on the potential risks of drug–drug interactions (DDIs) associated with the consumption of dietary polyphenols concurrently with chemotherapy treatment involving Pgp substrate/inhibitor drugs.

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**Heme oxygenase-1 in Efferocytosis**

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Heme oxygenase (HO-1) plays a vital role in the catabolism of heme and yields equimolar amounts of biliverdin, CO and free iron. We report that macrophages engulfing either the low amount of heme containing apoptotic thymocytes, or the high amount of heme containing eryptotic red blood cells (eRBCs) strongly upregulate HO-1. The induction by apoptotic thymocytes is fully dependent on soluble signals, which do not include adenylate cyclase activators, while in the case of eRBC-s it is cell uptake-dependent. Both pathways might involve regulation of BACH1, the repressor transcription factor of the HO-1 gene. Long-term continuous efferocytosis of apoptotic thymocytes is not affected by the loss of HO-1, but that of eRBCs is inhibited. This later is related to an internal signaling pathway that prevents the efferocytosis-induced increase in Rac1 activity. While uptake of apoptotic cells suppressed the basal pro-inflammatory cytokine production in wild type macrophages, in the absence of HO-1 engulfing macrophages produced enhanced amounts of pro-inflammatory cytokines. Our data demonstrate that HO-1 is required for both the engulfment and the anti-inflammatory response parts of the efferocytosis program.

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# Section 8

## Daprodustat accelerates elevated phosphate-induced osteogenic trans-differentiation of vascular smooth muscle cells and aorta calcification

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**Background:** Chronic kidney disease (CKD) is frequently associated with anemia, partially due to the insufficient production of erythropoietin. This condition is treated with recombinant human erythropoietin, however in advanced CKD its application increases the risk of cardiovascular events. These findings initiated search for new treatment options to target the hypoxia inducible factor 1 (HIF1) pathway. Daprodustat (DPD) is a prolyl hydroxylase inhibitor which is approved to treat anaemia of CKD patients in Japan. Recent studies revealed that hypoxia contributes to vascular calcification via the activation of HIF1 pathway,

therefore we addressed the pro-calcifying effect of DPD on vascular smooth muscle cells (VSMCs).
**Methods:** We induced calcification of human VSMCs with phosphate (Pi, 1.5-2.5 mmol/L) in the presence or absence of DPD (10-1000 nmol/L). Protein expressions of hypoxia targeted genes, ER stress markers and osteogenic differentiation markers were evaluated by Western blot and ELISA. Extracellular matrix mineralization was assessed by Alizarin red staining and Ca measurement. Knockdown of ATF4 and HIF1was carried out by silencing RNA technology. We applied aorta ring culture model and adenine-induced CKD model in C57BL/6 mice to study the effect of DPD on aorta calcification under *ex vivo* and *in vivo* conditions respectively.
**Results:** As expected, DPD increased HIF-1α, VEGF and Glut-1 protein expressions in VSMCs in a dose-dependent manner. DPD induced protein expressions of osteogenic and ER stress markers and potentiated the effect of Pi on extracellular matrix calcification in VSMCs. Knockdown of ATF4 (ER stress-associated transcription factor) similarly to HIF1α decreased the DPD-induced matrix mineralization. DPD increased Pi-induced calcification of mice aorta rings under *ex vivo* conditions. Oral administration of DPD in mice with CKD corrected anaemia but elevated the Ca content of the aorta.
**Conclusions:** We concluded that DPD increases Pi-mediated osteogenic trans-differentiation and extracellular matrix mineralization of VSMCs. Our data suggest the involvement of ER stress via ATF4 signalling pathway as a potential underlying mechanism in Pi- and DPD-induced calcification of VSMCs. Our *ex vivo* and *in vivo* results show the pro-calcifying effect of DPD in mice aorta.

## THE ROLE OF NEUTROPHIL GRANULOCYTES IN SKELETAL MUSCLE INFLAMMATION AND REGENERATION

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**Introduction:** The skeletal muscle is permanently exposed to physical damage but in normal condition the tissue is able to repair itself in a very efficient way. Neutrophils enter traumatized tissue firstly and act as professional phagocytic cells. Like macrophages, neutrophils also form a heterogeneous population, they can contribute to inflammation and to repair as well. The myeloid-specific deletion of Mcl-1 anti-apoptotic protein in mice leads to dramatic reduction of neutrophil counts. In these mice we observed a delayed skeletal muscle regeneration. Our aim is to investigate the role of neutrophils during a sterile muscle injury in order to understand the molecular mechanisms and immunological pathways better in tissue regeneration.
**Methods:** The muscle injury is induced by cardiotoxin (CTX) injection in tibialis anterior muscle. The muscle isolations and processing are performed on different consecutive days after injury. The regeneration is monitored by analysing satellite cells, fibro/adipogenic progenitors (FAPs) and infiltrating immune cells with flow cytometry.
**Results:** The phenotypic analysis of wild type (WT) and Mcl-1 KO mouse strains showed splenomegaly in Mcl-1-KO mice and increased number of myeloid progenitor

cells in the bone marrow and spleen. We compared two types of experimental models (CTX and barium-chloride injection) for muscle regeneration. The CTX-injury induced inflammation was associated with eosinophilic infiltration. This prolonged eosinophil migration can be detected for several days in Mcl-1 KO mice. The numbers of inflammatory Ly6C$^+$ monocytes and resolving F4/80$^+$ macrophages were reduced in Mcl-1 KO mice compared to WT mice. The muscle regeneration was followed by measuring the FAPs and satellite cells with flow cytometry. The high number of these cells at day 7 post injury in Mcl-1 KO mice may refer to delayed regeneration.
**Conclusion:** These preliminary results show that the myeloid-specific deletion of Mcl-1 anti-apoptotic protein affects the neutrophil and monocyte/macrophage counts too. In the absence of neutrophils the muscle regeneration takes more time than in normal condition. Next, we would like to restore the delayed repair with transferring neutrophils from MHC-compatible mice by adoptive cell transfer.
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**Investigating the Role of TAM kinase signaling in Muscle Regeneration**

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**Abstract:** Skeletal muscle regeneration following injury results from the proliferation and differentiation of myogenic stem cells, called satellite cells, located beneath the basal lamina of the muscle fibers. Infiltrating macrophages play an essential role in the process partly by clearing the necrotic cell debris, partly by producing cytokines that guide myogenesis. Infiltrating macrophages are at the beginning pro-inflammatory, but phagocytosis of dead cells induces a phenotypic change to become M2 (anti-inflammatory/healing) macrophages that regulate inflammation, fibrosis, vascularization and return to homeostasis. The TAM receptor kinases Mer and Axl are known phagocytic receptors in macrophages functioning in tolerogenic or inflammatory conditions, respectively. Here we investigated their involvement in the muscle regeneration process by studying the muscle repair following cardiotoxin-induced injury in Mer null mice. We found that Axl was the dominant TAM kinase receptor expressed by both skeletal muscle cells and early-infiltrating macrophages, but the expression of macrophage Mer significantly increased during repair. Mer ablation did not affect the skeletal muscle weight or structure, but following injury it resulted in a delay in the clearance of necrotic muscle cell debris, in the M2 phenotype conversion of macrophages and consequently in the full muscle regeneration process. Administration of the TAM kinase inhibitor BMS-777607 into wild type mice mimicked the effect Mer ablation on the muscle regeneration process, but additionally it has no effect on the myoblast fusion as well. Finally, in vitro inhibition of TAM kinase signaling in C2C12 myoblast cells resulted reducing the relative myosin protein content which indicate impaired in muscle regeneration. Taken together these findings reveal the contribution of TAM kinase-mediated signaling to the skeletal muscle regeneration both in macrophages and in myoblasts.

**Role of retinol saturase enzyme in skeletal muscle regeneration**

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**Abstract:** Apoptosis and proper clearance of apoptotic cells play a central role in maintaining tissue hemostasis. Skeletal muscle regeneration is a complex process orchestrated by multiple steps. The clearance of dead cells and the tightly orchestrated inflammatory response subsequent of the tissue injury are crucial in the initiation of muscle reparation. Retinol saturase (retsat) is an oxidoreductase enzyme expressed in metabolically active tissues and it has been implicated in macrophage function as they display decreased phagocytic capacity.
The aim of our study was to investigate the impact the loss of retinol saturase (retsat) on *in vivo* skeletal muscle regeneration. Muscle injury was induced by injecting snake venom cardiotoxin (CTX) in the tibialis anterior muscle in retsat+/+ (WT) and retsat-/- (KO) mice. Muscles were collected 2, 3, 4, 10 and 22 days post-injury for histological analysis, gene expression measurements and flow cytometry. Fluorescent staining with anti-laminin antibody and subsequent image analysis were performed to determine individual muscle fiber size in control and 10 and 22 days-old injured muscles.
Flow cytometric analysis of CD45+ cells isolated from injured muscles showed no difference in the ratio of macrophages and neutrophils between the two strains. The number of macrophages peaked at day 4 post injury while the highest number of neutrophils was detected at day 2 after injury in the both WT and KO mice. The size of control and regenerating fibers was also similar between WT and KO mice. Quantification of necrotic area in H&E stained muscle section showed higher percentage of necrotic area at day 10 post-injury in KO muscles as compared to WT ones. Quantitative analysis of pro-inflammatory cytokines (TNF, IL1b, IL6) and anti-inflammatory cytokines (IL-10, Tgfb1) for total muscle isolated from the regenerating muscles and quantification of multinucleated fibers to determine fiber fusion efficiency are in progress. The delayed clearance of necrotic cells might indicate potential role of retsat enzyme in skeletal muscle homeostasis.