15th Molecular, Cell and Immune Biology Winter Symposium

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

Debrecen, 6-7 January, 2022
Online Conference

15th Molecular, Cell and Immune Biology Winter Symposium

Programme:

January 6

9:00-10:55 Section 1 Chair: András Szabó
Link: https://unideb.webex.com/unideb/j.php?MTID=m5b3539b26daaeb48dbcf1a579a9e6c8b

Opening

9:00-9:05 József Tőzsér

Plenary lectures

9:05-9:40 Zoltán Szekanecz:
Immunological aspects of COVID-19: pathogenesis, treatment and vaccination

9:40-10:05 Mohamed Mahdi:
Severe acute respiratory syndrome coronavirus 2: Susceptibility to repurposed antivirals, cellular transcriptomic changes induced by its spike protein, and analysis of vaccine-induced neutralizing antibodies

Introductory talk

10:05-10:25 Márton Miskei:
A misleading way to map binding sites of the transposase-derived SETMAR protein in the human genome

Regular talks

10:25-10:40 Csaba Fillér:
COMPASS histone modification complex as a key player of DNA double-strand break (DSB) formation in meiotic S. cerevisiae cells

10:40-10:55 Gyula Hoffka:
Theoretical studies on the underlying mechanism of enzyme evolution

10:55-11:15 Break

11:15-12:35 Section 2 Chair: János Mótyán

Link: https://unideb.webex.com/unideb/j.php?MTID=m4bf7d3a6dafd489bcf6440dfab4c7179

Introductory talk

11:15-11:35 András Szabó
Proteases in chronic pancreatitis

Regular talks:

11:35-11:50 Kiarie Irene Wanjiru:
Inhibition profiling of integrase strand transfer inhibitors against HIV-2

11:50-12:05 Zsuzsa Csobán-Szabó:
An attempt to reveal the biological function of human transglutaminase 4 in the intestinal tract

12:05-12:20 Ádám Diós:
Primary immune response of CeD risk infants predominantly targets non-deamidated epitopes of gliadin, while manifest patients' antibodies prefer deamidated epitopes when anti-TG2 autoantibodies emerge

12:20-12:35 Nastaran Tarban:
Investigation of the role of adenosine A3 receptor in skeletal muscle regeneration in mice

12:35-13:15 Lunch

13:15-14:40 Section 3 Chair: Kitti Pázmándi

Link: https://unideb.webex.com/unideb/j.php?MTID=mfaa2d0150dbd908765f4927e2e9f3668

Introductory talk

13:15-13:40 Zsolt Czimmerer:
The epigenetic state of IL-4-polarized macrophages enables activated NFkB-p65 cistrome expansion and extended synergistic responses to LPS/TLR ligands resulting in hyperinflammatory gene expression

Regular talks

13:40-13:55 Apolka Domokos:
Regulation of Vegfa expression and secretion in IL-4-polarized macrophages

13:55-14:10 Noemí Caballero-Sánchez:
Role of the transcription factor BACH1 in macrophage phenotype switch during tissue repair and regeneration

14:10-14:25 Dóra Bencze:
Investigating the NLRP3 pathway activity of human plasmacytoid dendritic cells in a high IFN signature-associated autoimmune disease

14:25-14:40 Hajnalka Emese Halász:
Characterization of neutropenic mouse model phenotype and sterile inflammation in them

14:40-15:00 Break

15:00-17:00 Section 4 Chair: Károly Jambrovics
Link: https://unideb.webex.com/unideb/j.php?MTID=m0c1fd6a33f6098980a6dcf84f4c6e440

15:00-15:15 Zsuzsanna Kolostyák:
EGR2 is a direct regulator of pathogen elimination-related transcriptional network in alveolar macrophages

15:15-15:30 Abhirup Shaw:
Irisin stimulates the release of CXCL1 via upregulation of NFκB pathway from human neck derived differentiating adipocytes

15:30-15:45 Eduárd Bíró:
Expression of intracellular pattern recognition receptors in skeletal muscle.

15:45-16:00 Lengyel Máté:
ZG16B, a rexinoid-regulated secreted factor increases mammary epithelial cell proliferation and migration

16:00-16:15 László Sós:
Palmitate inhibits efferocytosis by activating the mTORc1/RhoA/Rho kinase pathway

16:15-16:30 Kipchumba Biwott:
The role of the ABCB1 protein in cytotoxic lymphocytes

16:30-16:45 Hamidreza Mianesaz:
Signal and cell type dependent role of cis regulatory elements in cytokine expression regulation and cells programming

16:45-17:00 Zsuzsanna Szűcs:
An ultrarare manifestation of an X-linked recessive disorder: Duchenne dystrophy in a female patient

January 7

9:00-10:35 Section 5 Chair: Endre Kristóf
Link: https://unideb.webex.com/unideb/j.php?MTID=m922ee779df9354d7f51ae372bc143187

Introductory talk

9:00-9:20 Károly Jambrovics:
ATRA and ATO combination has several beneficial effects in treatment of Acute Promyelocytic Leukaemia cells

Regular talks

9:20-9:35 Alexandra Varga:
Study the role of miR-30 family members in the response of ovarian cells to high dose estrogen treatment

9:35-9:50 Sham Jdeed:
ARID1A mediates the anti-proliferative effects of bexarotene and carvedilol combination treatment in normal and transformed breast epithelial cells

9:50-10:05 Miklós Laczik:
Antidiabetics against cancer: metformin inhibits tumor growth by activating cohesin, interleukin 6, potentially affecting chromatin structure

10:05-10:20 Noemí Caballero-Sánchez:
Role of the transcription factor BACH1 in macrophage phenotype switch during tissue repair and regeneration

10:20-10:35 Dóra Géczi:
Analysis of circulating miRNA profile in plasma samples of glioblastoma patients

10:35-11:00 Break

11:00-12:30 Section 6 Chair: Bálint Bálint
Link: https://unideb.webex.com/unideb/j.php?MTID=mf9deb27d09f1ba093e927631346fa44d

11:00-11:15 Rini Arianti:
High Level of Thiamine is Required for Efficient Thermogenesis in Human Neck Adipocytes

11:15-11:30 Attila Vámos:
Mitophagy is upregulated during beige to white transition of human subcutaneous abdominal adipocytes

11:30-11:45 Boglárka Ágnes Vinnai:
Long-Term Thiamine Treatment Promotes Thermogenic Competency of Human Adipocytes

11:45-12:00 Máté Dávid Csiki:
The effect of alpha-ketoglutarate on phosphate and hypoxia-induced calcification of vascular smooth muscle cells

12:00-12:15 Haneen Muntasir Ababneh:
Dexamethasone and high glucose induce osteogenic differentiation of human lens epithelial cells

12:15-12:30 Arpan Chowdhury:
Activation of Nrf2/HO-1 axis by heme attenuates calcification of human lens epithelial cells

12:30-13:15 Lunch

13:15-14:30 Section 7 POSTER SESSION Chair: Róbert Király
Links:
Discussion:
https://unideb.webex.com/unideb/j.php?MTID=m8069925ecc21526ec3343d0e027e3939
Poster viewing:
https://drive.google.com/drive/folders/1-JQJ-IORbaEIdKOvFUe8xSJDcLWYhLR2?usp=sharing

Petra Magdolna Bertalan: DIA spectral libraries for salivary biomarkers to identify oral malignancies

Bianka Csaholczi: Development and characterisation of a transglutaminase 2 knocked down endothelial cell model

Dóra Csige: Investigation of the conformational epitope of mouse monoclonal antibody 885 recognizing a celiac-related surface in transglutaminase 2

Fatime Grimplinyi: Comparative analysis of transcriptomic changes induced by xenoestrogens in ovarian cells

Nathalí Haro: Optimization of the myeloid blood differentiation from pluripotent embryonic stem cells via embryoid body formation

Emiliána Jex: Impaired NLRP3 pathway activity in virus exposed human plasmacytoid dendritic cell

Sogol Khatmi: Functional characterization of two PNLIP variants identified in patients with pancreatic lipase deficiency

János András Mótyán: Purification of Schistosoma japonicum GST with metal ion affinity chromatography

Nikolett Németh: Cell-free nucleic acids and their application in diagnosing glioblastoma multiforme

Anna Anita Rácz: Invasive aspergillosis specific miRNA signatures in oncohematology patients

Eszter Anna Váradi: Zymosan-induced immune response in alternatively polarized macrophages

14:30-16:05 Section 8 Chair: Szilágyi-Bónizs Melinda
Link: https://unideb.webex.com/unideb/j.php?MTID=md0ef32dd5e676a9d1741c7cbdfb5ddca

Introductory talk

14:30-14:50 Gergő Kalló:
What is beyond protein identification? Opportunities for targeted and data-independent protein analysis

Regular talks

14:50-15:05 Tamás Linkner:
Analysis of the cellular proteo-transcriptomic changes following HIV-1/2 transduction in HEK-293T cells

15:05-15:20 Balázs Kunkli:
High throughput wine vinegar metabolite profiling by UPLC-Orbitrap Fusion Tribrid MS and programmatic annotation: A methodological approach

15:20-15:35 Ajnees Kumar:
Network analysis of atheroma and complicated lesions in human atherosclerosis

15:35-15:50 Erdenetsetseg Nokhoijav:
Proteomics and metabolomics profiling of serum from patients with obesity or type 2 diabetes

15:50-16:05 László Madar:
Establishing the mutational spectrum of Hungarian patients with familial hypercholesterolemia

16:05 Concluding remarks
József Tőzsér (Chair), Gábor Szabó, László Fésüs, István Balog, Szilvia Benkő, Bálint Nagy, Zsuzsa Szondy

ABSTRACTS

Section 1

Severe acute respiratory syndrome coronavirus 2: Susceptibility to repurposed antivirals, cellular transcriptomic changes induced by its spike protein, and analysis of vaccine-induced neutralizing antibodies

Mohamed Mahdi1, Noémi Miltner1, Aya Almuffti1,2, Tamás Linkner1,2, Viktor Attila Ambrus1,2 and József Tőzsér1
1Laboratory of Retroviral Biochemistry, Department of Biochemistry and Molecular biology, University of Debrecen, Faculty of Medicine, Debrecen, Hungary
2Molecular Cell- and Immune Biology Doctoral School, University of Debrecen, Faculty of Medicine, Debrecen, Hungary

The new coronavirus variant SARS-CoV-2 is the causative agent of COVID-19 pandemic that has infected almost 300 million worldwide and resulted in more than 5 million deaths. Two years after the start of the pandemic, neither a standardized treatment protocol, nor antiviral treatment of proven efficacy has been established to date for COVID-19, however, a variety of vaccine types based on the expression of a full, or partial SARS-CoV-2 spike protein are being developed and distributed, in an attempt to curb the pandemic. We set out to analyze the efficacy of repurposed potential antivirals targeting the viral main protease (Mpro) and RNA-dependent polymerase (RdRp), utilizing in vitro and cell culture-based methodologies. Also, given the widespread use of urgently approved mRNA-based vaccines targeting the viral spike protein, and being an RNA virus, the potential of SARS-CoV-2 to adapt in order to evade the neutralizing antibodies remains high, hence, we began characterizing the development of neutralizing antibodies in post-vaccinated Hungarian individuals, and carried out cell culture-based neutralization assays utilizing pseudovirions enveloped with different SARS-CoV-2 spike variants, primarily the prototypical Alpha, and Beta variants, while analysis of other variants of concern is still ongoing. We hereby briefly present our findings in regards to vaccine-induced neutralizing antibodies, and delineate the cellular transcriptomic changes induced by transfection of the SARS-CoV-2 spike protein in human macrophages.

This work was supported by the Thematic Excellence Programme TKP2021-EGA-20 (Biotechnology) of the Ministry for Innovation and Technology in Hungary

A misleading way to map binding sites of the transposase-derived SETMAR protein in the human genome

Márton Miskei1, Adrienn Horváth1, Éva Nagy1, Lóránt Székvölgyi1,2
1 MTA-DE Lendület 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

SETMAR is a naturally occurring fusion protein that consists of a histone-lysine methyltransferase domain and an HsMar1 transposase. To elucidate the biological role of

SETMAR, it is crucial to identify genomic targets to which SETMAR specifically binds. We managed to map the genomic landscape of the SETMAR in a near-haploid human leukemia cell line (HAP1). Our analysis revealed a perfect correlation between SETMAR and inverted tandem repeats (ITRs) of HsMar1 transposon remnants, which are considered as natural target sites for SETMAR chromosome binding. However, we did not detect any untargeted events at non-ITR sequences, calling into question previously proposed off-target binding sites. We identified sequence fidelity of the ITR motif as a key factor for determining the binding affinity of SETMAR for chromosomes, as higher ITR fidelity resulted in increased affinity for chromatin and stronger repression of SETMAR-bound gene loci. In this presentation I will highlight a common trap in the ChIP-seq method that potentially misleads researchers when analyzing the results. I will show that effective analysis of ChIP-seq data requires sufficient coverage of NGS sequence reads.

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Reference:
Miskei M et al. 2021. Genome-wide mapping of binding sites of the transposase-derived SETMAR protein in the human genome. Comput Struct Biotechnol J 19: 4032–4041. https://doi.org/10.1016/j.csbj.2021.07.010.

COMPASS histone modification complex as a key player of DNA double-strand break (DSB) formation in meiotic S. cerevisiae cells

Csaba Fillér1, Dóra Szabóné Varga1, Adrienn Horváth1, Orsolya Feró1, Beáta Boros-Oláh1, Lóránt Székvölgyi1,2
1 MTA-DE Lendület 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

: Double-strand DNA breaks (DSBs) occur during meiosis I at particular genomic sites called hotspots, that in Saccharomyces cerevisiae are located in intergenic regions of the genome (4). An important element required for the initiation of meiotic recombination is histone H3 lysine 4 trimethylation (H3K4me3), marking meiotic DSB sites (3). This process requires two key proteins of the Set1C/COMPASS histone modification complex: Set1 and Spp1 (4). Set1 is the histone methyltransferase that catalyzes the mono-, di-, and tri-methylation of histone H3 at lysine 4, and the Spp1 regulates the tri-methylase activity of Set1 through its 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 remains unknown.
Method: To detect all intra- and interchromosomal interactions in the genome of meiotically differentiating yeast cells we applied a high-resolution chromosome conformation capture methodology called Hi-C. Our investigation was carried out in wild-type and mutant yeast strains (spp1Δ, set1Δ and mer2sID (sID: Spp1 interaction-deficient) at multiple meiotic timepoints. NGS was performed on two biological replicates at a depth of ~20 million reads per time point, allowing high spatial resolution and efficient bioinformatic analysis.
Results: In the absence of functional Spp1 and Set1 H3K4me3- and DSB levels are greatly reduced (1, 4) and therefore loop-axis tethering is expected to be affected in the prophase of meiosis, when DSBs normally form. Accordingly, Hi-C results obtained with spp1Δ, set1Δ

and mer2sID mutants revealed a remarkable dysfunction in chromatin loop formation during meiosis, with decreased levels of intra- and interchromosomal (centromere-centromere) interactions.

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-21-5-DE-11 new national excellence program of the Ministry For Innovation and Technology from the source of the National Research, Development and Innovation Fund.

References:

1. Acquaviva, L. & Székvölgyi, L., et al..: The COMPASS subunit Spp1 links histone methylation to initiation of meiotic recombination. (2013) Science, 339 (6116), pp. 215-218.

2. Karányi, Z. et al.: 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. et al. 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.

Theoretical studies on the underlying mechanism of enzyme evolution

Gyula Hoffka1,2, Mónika Fuxreiter1,3

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3Department of Biomedical Sciences, University of Padova, Italy

The design of artificial enzymes is one of the greatest challenges facing biotechnology. Successful designs, with activities comparable to naturally occuring enzymes, could have revolutionary industrial applications. To date, the most successful artificial enzyme is the HG-3.17 Kemp eliminase, which was created with combined computational design and directed evolution experiments.

We have applied multiscale simulations to better understand the evolutionary driving forces leading to HG-3.17. The multiscale Empirical Valence Bond method was used as the framework of our studies, and the calculations were carried out using the Free Energy Perturbation/Umbrella Sampling techniques. Besides the enzymes, we have also simulated the reference reaction in water, to calibrate our method. The results were validated with comparison to the experimental activation energies.

We have examined the different conformers of both monomers found in the crystal structures. Our studies show that π-stacking interactions between the substrate and the Trp44 residue are crucial for the reaction. We have compared the activities of ensembles with both conformers of Trp44 to the experimental data, showing that our results are also in good agreement with further crystal structure studies.

Different types of contributions to the reaction were also calculated for the amino acid residues, highlighting the favorable role of the mutations. We have also compared the interaction networks in both monomers, and found that as the evolution progresses, the networks share an increasing number of matching interactions.

Section 2

Proteases in chronic pancreatitis

András Szabó
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Chronic pancreatitis is a severe inflammatory disorder of the human pancreas characterized by permanent destruction of the pancreatic parenchyma, inflammatory cell infiltration, fibrosis and calcifications within the excretory ducts. The disease may lead to exocrine insufficiency and malabsorption, disabling abdominal pain, diabetes mellitus and pancreatic cancer.

Chronic pancreatitis frequently develops on the basis of increased genetic risk associated with mutations in genes of pancreatic digestive proteases such as PRSS1, CTRC, CPA1 and the secretory protease inhibitor SPINK1. Pancreatic proteases are secreted by acinar cells as inactive precursors and activated by proteolytic cleavages upon reaching the intestines. Premature intrapancreatic protease activation may cause auto-digestion of the pancreas and the development of chronic pancreatitis. Trypsin is a master proteolytic regulator of pancreatic protease activation. On the one hand, trypsin is capable of auto-activation and, on the other hand, it activates other digestive proteases. Due to these properties, trypsin activation is tightly regulated in the pancreas. Chymotrypsin C (CTRC) is a minor pancreatic chymotrypsin isoform, which limits intrapancreatic protease activation by degrading trypsin. Cationic trypsin encoded by the PRSS1 gene is one of the major genetic risks of chronic pancreatitis. Gain-of-function mutations in PRSS1 cause increased intrapancreatic protease activation due to reduced CTRC-mediated proteolysis. SPINK1 is a specific secretory trypsin inhibitor of the human pancreas, which protects the pancreas against premature trypsin activation. Consequently, loss-of-function mutations in both CTRC and SPINK1 increase the risk for chronic pancreatitis. Recently, an alternative pathomechanism unrelated to increased trypsin activation has been described. Mutations causing protein misfolding and endoplasmic reticulum stress in highly expressed genes such as CPA1, encoding human carboxypeptidase A1, and PRSS1 were strongly associated with chronic pancreatitis.

Taken together, mutations in genes expressed in the human pancreas exert their pathogenic effect through the dysregulation of intrapancreatic trypsin activation or mutation-induced protein misfolding and consequent endoplasmic reticulum stress in pancreatic acinar cells.

Inhibition profiling of integrase strand transfer inhibitors against HIV-2

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2Molecular Cell- and Immune Biology Doctoral School, University of Debrecen, Faculty of Medicine, Debrecen, Hungary

The ability to incorporate a copy of viral genome into the host cell is a very crucial step in the life-cycle of retroviruses. HIV-2 integrase is a 32 kDa enzyme that catalyzes the integration of

proviral DNA into the host cellular genome, and hence, is an important therapeutic target. Integrase strand transfer inhibitors (INSTIs) are crucial pillars in the treatment of HIV infection, and are recommended as first-line regimens given their high efficacy and tolerability. Current INSTIs are primarily designed for treatment of HIV-1 infection, and their efficacy against HIV 2 remains widely understudied and inconclusive, supported only by few limited phenotypic susceptibility studies. We therefore carried out inhibition profiling of a panel of INSTIs against HIV-2 in cell culture, and determined the IC50 of the inhibitors in comparison to HIV-1. Our results indicate that while the inhibitors showed a good efficacy against HIV-1, as reported previously, most of them failed to effectively inhibit integration of HIV-2. To confirm our findings, we are carrying out in vitro inhibition assays utilizing purified HIV-2 integrase, results of which are still ongoing. Nevertheless, our preliminary findings are novel, and shed light on the use of INSTIs in the context of infection by HIV-2.

This work was supported by the Thematic Excellence Programme TKP2021-EGA-20 (Biotechnology) of the Ministry for Innovation and Technology in Hungary

An attempt to reveal the biological function of human transglutaminase 4 in the intestinal tract

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Keywords: transglutaminase 4, saliva, protein-protein interaction, proteomics

Transglutaminase 4 (TG4) is one of the less-studied member of the transglutaminase enzyme family. It was described in rodents responsible for the formation of a viscous copulatory plug, but the human functions of the protein are poorly studied. TG4 is expressed mainly in the prostate and regulates semen viscosity and immunogenicity of sperm cells. It is also involved in cancer invasiveness and autoimmune diseases. Our goal was to study the presence and potential role of TG4 in the intestinal tract.

To confirm the extra-prostatic expression of TG4, we reanalysed proteomics databases and found that TG4 is present in the colon, heart, seminal plasma, prostate, seminal vesicle, spermatozoon, spleen, urinary bladder, and salivary glands as well. TG4 secretion into the saliva was confirmed by western blot, but its identification by mass spectrometry (LC-MS/MS) has not been successful yet. In the extracellular vesicle (EV) fraction of the saliva, we enriched the full-length form of TG4, but this was still not sufficient for LC-MS/MS identification.

In order to reveal the biological function of TG4 in human saliva, a protein-interacting partner search was performed using site-specifically biotinylated recombinant human TG4. After Neutravidin agarose separation, the bound proteins were analysed by LC-MS/MS. 292 potential interaction partners were identified. Based on bioinformatics analysis, the most enriched “Biological Processes” and “Molecular Functions” were immune regulatory and secretory functions, calcium-dependent phospholipid binding, enzyme binding, enzyme inhibitor activity and lipid binding. The data will be confirmed by biolayer-interferometry measurement.

In order to gain information about TG4 substrate preference, potential TG4 substrates were identified in saliva and AD-293 epithelial cells by a biotin-pentylamine incorporation assay. After NeutrAvidin separation, the samples were analysed by LC-MS/MS. In saliva, only a few proteins were identified as TG4 substrates, for example, 14-3-3 protein isoforms, cystatin-SN, and the immunoglobulin heavy constant alpha 1. In AD-293 cells, among several potential TG4 substrate proteins, 20 peptides were identified with BPA modification, but no consensus sequence could be obtained. Many of the identified cellular TG4 substrates are involved in cell-cell interaction, adhesion and proliferation, which raises the possibility of targeting TG4 in anticancer therapy.

This study was supported by the National Research, Development and Innovation Office of Hungary (NKFIH-K120392, K129139).

Primary immune response of CeD risk infants predominantly targets non-deamidated epitopes of gliadin, while manifest patients' antibodies prefer deamidated epitopes when anti-TG2 autoantibodies emerge

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3 Department of Pathophysiology and Allergy, Division of Immunopathology, University of Vienna, Vienna, Austria
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Celiac disease (CeD) is a conditional autoimmune disorder triggered by the consumption of gluten prolamins in genetically susceptible subjects. CeD is characterized by the coupled T-cell mediated immune response to gliadin (a wheat gluten prolamin) and self-protein tissue transglutaminase (TG2). TG2 contributes to CeD pathomechanism by posttranslationally modifying gliadins, since deamidated gliadin peptides are more immunogenic. The appearance of anti-gliadin antibodies precedes the autoantibody production, but we have limited knowledge about the disease triggering anti-gliadin immune response in the pre-diagnosis phase. We took advantage of a prospective dietary intervention study, where infants with high CeD risk underwent double blind placebo controlled gluten challenge and regular blood testing. With the help of the prospectively collected serum samples we can follow the changes in anti-gliadin immune response related to disease manifestation. We found that infants in response to the primary gluten introduction produced high affinity anti-gliadin antibodies, which preferably targeted non-deamidated gliadin epitopes. The appearance of IgA isotype antibodies was also detected, which cannot have maternal origin, hence indicates infants' own adaptive immune response to the ingested gliadin. In contrast to that, anti-gliadin antibodies produced at the time of CeD manifestation displayed preference shift towards the deamidated gliadin epitopes and affinity maturation toward the deamidated PEQPFP motif. The emerging autoantibodies produced by manifest patients predominantly targeted the epitope 2 surface of human TG2

enzyme. Our results reveal that primary immune response preferably targets the non-deamidated epitopes, while at the time of disease manifestation, the deamidated epitopes of gliadin and the deamidating enzyme, TG2 come in the crosshair of the immune system.

The study was supported by NKFI 120392, EFOP-3.6.1-16-2016-00022 and Interreg DTP571 CD SKILLS.

Investigation of the role of adenosine A3 receptor in skeletal muscle regeneration in mice

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Skeletal muscle regeneration is initiated by local inflammation and is accompanied by the removal of necrotic myofibers from the injured area. This regeneration is composed of three major phases: first, inflammation characterized by leukocyte infiltration to the damage site, second, new tissue formation where quiescent satellite cells (SCs) become activated and differentiate into myoblasts which fuse together, and third, remodeling phase when the original muscle architecture is restored. Apoptotic cell phagocytosis regulates the inflammatory program in macrophages (MFs) by converting the M1-like inflammatory phenotype into M2-like healing one. This timed switch is orchestrating the regeneration process and therefore the abnormal phagocytosis or deregulated inflammation can lead to impaired muscle regeneration. The necrotic cells release the intracellular ATP as a danger signal. ATP is then degraded to adenosine which activates the purinergic receptors concentrated at the leading edge of the MFs to guide MF chemotactic migration toward the damage site. Previously, we have shown that loss of adenosine A3 does not affect the direct phagocytotic capacity of MFs but impaired their chemotactic navigation ability and that its activation also negatively regulates the apoptotic cell engulfment-dependent suppression of inflammation. Therefore we decided to test the impact of adenosine A3 ablation on skeletal muscle regeneration.

Muscle injury was induced by cardiotoxin injection into the tibialis anterior of wild-type and A3 receptor null mice. Based on cross-sectional area measurements we found that the average fiber diameter was similar in the control muscles but the size of regenerating fibers and the ratio of multinucleated fibers were higher in the injured muscles of knockout mice. The number of infiltrating $CD45^+$ leukocytes was higher in the regenerating knockout muscles. We also detected a bigger population of $Ly6C^+$ M1-like MFs and a higher number of SCs during the early phase and lower collagen deposition at the later phase of regeneration in the knockout muscles. We also found that the expression of the myogenic markers Pax7, MyoD, and myogenin was increased in the knockout muscle compared to wild-type ones. Moreover, the expression of the M2 MF-specific GDF3, IL-10, and arginase 1 peaked earlier in the regenerating knockout muscles.

These results indicate that adenosine A3 receptor affects cytokine and growth factor expression in the regenerating skeletal muscle and loss of it results in accelerated inflammatory program and regeneration in the injured muscle.

Section 3

The epigenetic state of IL-4-polarized macrophages enables activated NFkB-p65 cistrome expansion and extended synergistic responses to LPS/TLR ligands resulting in hyperinflammatory gene expression

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Prior exposure to signals can fundamentally change the response of innate immune cells to subsequent stimuli underlying phenomena such as cellular memory, tissue type-specific reactions, cytokine storm, or anergy/tolerance. Our understanding of the molecular nature of such interactions, especially at the epigenomic and gene expression level, is fragmented. It is believed that alternative macrophage polarization and inflammatory signals-activated transcriptional programs largely antagonize each other, and no significant convergence has been identified between them. In contrast, here we show that IL-4 polarized macrophages establish a unique inflammatory gene expression program upon lipopolysaccharide exposure. This interaction, we termed extended synergy, is dependent on IL-4 -induced STAT6- directed epigenomic remodeling, the vast expansion of the LPS-activated NFκB-p65 cistrome, increased chromatin accessibility, and enhancer activity. The EGR2 transcription factor is required to enable the LPS-induced *de novo* and enhanced NFκB-p65 binding and synergistic gene activation. As a result of this, the previously alternatively polarized macrophages produce immune-modulatory factors, including CCL17, CCL22, CCL2, and EDN1, at an extremely high level *in vitro* and *in vivo* in a murine Th2-type airway inflammation model upon LPS exposure. Our findings thus establish that the IL-4-STAT6-EGR2 signaling pathway-induced epigenetic reprogramming is responsible for the development of robust inflammatory hyperresponsiveness to TLR activation and likely contributes to lung pathologies.

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Regulation of Vegfa expression and secretion in IL-4-polarized macrophages

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As a part of the innate immune system, macrophages participate in various physiological and pathological processes. One of the endpoints of macrophage polarization states is the Th2-type cytokines (Il-4 and IL-13) induced alternative or M2 polarization. The alternatively polarized macrophages are involved in wound healing, tissue regeneration, and protection against nematode infection. Besides, alternative macrophage polarization is also observed in various human diseases, including tumor development and progression, fibrosis, and allergic asthma. Although these diseases are often associated with pathological angiogenesis and uncontrolled pro-angiogenic factor VEGFA production, the potential angiogenesis-regulating role of alternatively polarized macrophages is controversial.
We aimed to study whether IL-4 can modulate the VEGFA producing capacity of murine bone marrow-derived macrophages at the transcriptional and post-transcriptional level under basal normoxic conditions or following pro-angiogenic stimuli such as hypoxia or RXR/RAR ligand activation. To investigate the Vegfa mRNA and secreted protein production and anti-angiogenic Vegfa decoy receptor Flt-1 expression, we applied RT-qPCR, ELISA, and Western blot methods.
We observed that IL-4 transiently repressed the basal and pro-angiogenic stimuli-induced Vegfa mRNA expression. IL-4 also highly induced the mRNA expression of Flt-1 under normoxic conditions. The hypoxia could further increase the IL-4-induced Flt-1 expression, while RXR ligand LG268 had a negative effect on the IL-4-dependent enhancement of Flt-1 expression. The STAT6 and EGR2 transcription factors play an important role in the regulation of both Vegfa and Flt-1 expression. Finally, we found that the secreted VEGFA protein content was dramatically decreased in the supernatants derived from IL-4 polarized macrophages.
Overall, our findings raised the possibility that IL-4 can enhance the anti-angiogenic activity of macrophages throughout the repression of pro-angiogenic Vegfa and the induction of anti-angiogenic Flt1 expressions. Still, the exploration of the exact mechanism requires further experimental work.

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Role of the transcription factor BACH1 in macrophage phenotype switch during tissue repair and regeneration

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After injury, circulating monocytes infiltrate the muscle and differentiate toward macrophages. At early time-points after the injury, the vast majority of infiltrating monocyte-derived macrophages present a pro-inflammatory phenotype, and later on there is a gradual transition from proinflammatory to anti-inflammatory phenotype. This process, named macrophage switch, is crucial for correct tissue repair; however, the chromatin-level mediators and regulatory events controlling this switch remain unknown. To delineate the timing of transcriptional changes, we used the cardiotoxin acute muscle injury model and we profiled chromatin accessibility and gene expression in sorted macrophage populations at different time-points of muscle regeneration. We identified a heme-binding transcriptional repressor, BACH1, as a novel regulator of this process. Bach1 knockout mice present an abnormal macrophage switch leading to impaired muscle regeneration and disrupting the expression of important genes involved in inflammation and tissue repair. To study how BACH1 affects the macrophage polarization during muscle repair we have performed Single Cell RNA-Seq experiments in WT and BACH1 LOF mice at different time points. These results show different clusters of macrophages depending on their gene expression pattern. Using WT / BACH1 LOF comparison will identify the magnitude of expression of critical inflammatory and repair-related genes.

INVESTIGATING THE NLRP3 PATHWAY ACTIVITY OF HUMAN PLASMACYTOID DENDRITIC CELLS IN A HIGH IFN SIGNATURE-ASSOCIATED AUTOIMMUNE DISEASE

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Introduction: As professional type I interferon (IFN) producing cells, plasmacytoid dendritic cells (pDCs) are the major coordinators of antiviral responses and participate in the pathogenesis of many autoimmune diseases. However, the NLRP3 dependent IL-1β pathway, which coordinates immune responses to non-viral infections and even autoimmune diseases, remained still unexplored in this cell type. Thus, our aim was to determine the functional activity of the IL-1β pathway and its potential interaction with the type I IFN pathway in pDCs derived from healthy individuals and psoriatic patients with high IFN signature.
Methods: Peripheral blood mononuclear cells were isolated from healthy individuals and psoriasis patients, then treated with distinct Toll-like receptor (TLR) agonists and specific NLRP3 activators. The activity of the NLRP3 pathway in the pDC population gated on BDCA-4 was analyzed by intracellular protein staining using flow cytometry.
Results: We observed that different TLR stimuli have various effects on the inflammasome activity of pDCs. Potent NF-κB inducers promoted higher levels of pro-IL-1β and nigericin-induced cleaved IL-1β production, compared to those activation signals, which mainly trigger IRF-mediated type I IFN induction. Furthermore, we showed that the presence of IFN-α significantly decreased the IL-1β production of pDCs, and vice versa pre-treatment with IL-1β

led to lower TLR-mediated type I IFN production. In line with these results, we also detected significantly lower IL-1β production in pDCs of psoriasis patients compared to healthy individuals. Since psoriasis is associated with high IFN-α levels, these findings further indicate that type I IFNs may inhibit NLRP3 inflammasome activity in pDCs.
Conclusion: Our results suggest that the NLRP3-dependent IL-1β secretory pathway is inducible in human pDCs, and can be inhibited by activating the type I IFN pathway. Generally reciprocal antagonistic effects can be observed between the antiviral type I IFN and the antibacterial IL-1β pathways, which not only affect antimicrobial responses, but also shape the immune responses in autoimmune diseases.
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Characterization of neutropenic mouse model phenotype and sterile inflammation in them

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Introduction: Neutrophil granulocytes are part of first defence of innate immunity against extracellular pathogens. They are mainly known by their role in inflammation and pathogen elimination (phagocytosis, degranulation and NETosis), but neutrophils are also multifaced immune cells. They contribute to resolution of inflammation too and can regulate the function of other immune cells by producing cytokines or by cell-cell interactions directly. Mcl 1 anti-apoptotic protein is indispensable in the development and survival of neutrophil granulocytes, so its myeloid-specific deletion leads to a severe neutropenia in mice.
Aims: The knock-out animal models make possible to investigate the function and the role of a gene or cell type in different processes. Our aim is to characterize phenotypically Mcl-1ΔMyelo mice for the well-founded investigation of the role of neutrophil granulocytes in sterile muscle inflammation and tissue regeneration in further experiments.
Methods: Besides the morphological observation of the mice, immune cells from bone marrow and spleen were isolated and analyzed by flow cytometry. Sterile inflammation was induced in tibialis anterior muscle by cardotioxin (CTX) injection. The muscle isolations and processing are performed on different consecutive days after injury. The inflammation and regeneration was monitored by analysing infiltrating immune cells, satellite cells (SCs) and fibro/adipogenic progenitors (FAPs) with flow cytometry.
Results: Erythropoiesis was significantly reduced in Mcl-1ΔMyelo mice bone marrow. Lower number of immune cells was observed too, but only the neutrophil count was significantly decreased. The most striking difference between the two mouse strains was the splenomegaly in neutropenic mice. Despite the spleen enlargement there was no difference in myeloid cell numbers. In the spleen of Mcl-1ΔMyelo mice, we detected elevated number of nucleated red blood cells (nu-RBC) and increased common myeloid progenitor (CMP) counts. An accumulated population of “defective” neutrophil cells can be observed both in the spleen and in the bone marrow.
As expected, neutrophil infiltration was barely observable in Mcl-1ΔMyelo mice during sterile inflammation, but CTX-induced injury was associated with increased eosinophil immigration.

The numbers of inflammatory Ly6C+ monocytes and resolving F4/80+ macrophages were reduced in neutropenic mice compared to WT mice. Interestingly, both αβ- and γδ-T cells were present in elevated number at the site of inflammation. The ratio of γδ T cells/αβ T cells is also higher in the inflamed tissue. The muscle regeneration can be followed by measuring the FAPs and SCs with flow cytometry. The high number of these cells at day 7 post injury in Mcl-1 defficient mice may refer to delayed regeneration.
Conclusions: The myeloid specific deletion of Mcl-1 gene leads to phenotypic changes in mice. Besides the neutrophil granulopoiesis, erythropoiesis is also affected in Mcl-1ΔMyelo mice. Splenomegaly in neutropenic mice may be due to a relocation of erythrocyte development, as evidenced by high CMP counts and the appearance of nu-RBCs in the spleen. We hypothesize that decreased neutrophil number rises the demand of increased neutrophil production. In parallel the erythroid compartment shrinks in the bone marrow. The spleen compensate the demand for erythrocytes.
In the absence of neutrophils an impaired inflammation and tissue regeneration can be observed. In further experiments, we would like to prove role of neutrophils in the regeneration process by adoptive cell transfer of neutrophils from MHC-compatible mice. Our further 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.

Section 4

EGR2 is a direct regulator of pathogen elimination-related transcriptional network in alveolar macrophages

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Alveolar macrophages (AMs) as dominant protective cells of lung innate immune system create protection against air pollutants and inhaled pathogens. The fundamental role of AMs is well known in different pulmonary pathologies. The study of transcriptional regulatory processes which direct the development and cellular functions of AMs is important in the identification of therapeutic strategies.
Early Growth Response 2 (EGR2) is a transcription factor which described as a late marker transcription factor of AMs, but its complex epigenetic role in macrophages has not well characterized yet.
In our study, we applied Egr2 LysozymeM-Cre mice for the examination of transcriptomic and epigenetic alterations in EGR2 deficient AMs. We performed RNA-sequencing and ATAC-sequencing experiments from AMs isolated by bronchoalveolar lavage to determine the alterations in transcriptome and in chromatin openness. Based on the bioinformatically integrated results of genome-wide approaches, we predicted the potential functional impairment in antifungal immunity. We performed ex vivo and in vivo experiments using zymosan and Aspergillus fumigatus treatment to prove our hypothesis. Based on our results the EGR2 deficiency leads decreased uptake and elimination of zymosan and Aspergillus fumigatus conidia, moreover the resolution of inflammation is longer after intranasal zymosan treatment.
Our findings indicate the transcriptional activator function of EGR2 in AMs and we identified this factor as a key direct regulator of pathogen elimination associated pathways and zymosan induced inflammation.

Irisin stimulates the release of CXCL1 via upregulation of NFκB pathway from human neck derived differentiating adipocytes

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Obesity can be considered as a 21st century epidemic with its global prevalence almost tripled since 1970. Thermogenic brown and beige adipocytes function in utilizing excess fat to produce heat by thermogenesis, thereby counteracting obesity. Recent studies in rodents and humans have indicated that these adipocytes also release cytokines, termed “batokines” that may play a vital role in whole body energy homeostasis. Irisin was discovered as a polypeptide regulator of beige adipocytes primarily released by myocytes during exercise.
We intended to characterize how irisin affected differentiating adipocytes derived from human subcutaneous neck (SC) and deep neck (DN) adipose tissue depots. Preadipocytes were isolated from SC and DN biopsies of the same donor, followed by differentiation with a white adipogenic differentiation protocol in the presence or absence of irisin. Global gene expression analysis was performed by RNA-sequencing on nine independent donor biopsies.
Irisin prevented the upregulation of characteristic thermogenic genes, such as UCP1, while upregulated genes belonging to various cytokine signaling pathways. Out of the several upregulated cytokines, CXCL1 (the highest upregulated) was found to be released throughout the entire differentiation period, predominantly by SC and DN derived differentiating adipocytes. Deep neck area tissue biopsies also showed a significant release of CXCL1 during 24 hours irisin treatment. Gene expression data indicated upregulation of the NFκB pathway upon irisin treatment, which was validated by an increase of p50 and decrease of IκBα protein level, respectively. Continuous blocking of the NFκB pathway by SN50 (cell permeable

inhibitor of NFκB nuclear translocation) significantly reduced the release of CXCL1. The released CXCL1 exerted a positive effect on the adhesion capability of endothelial cells.
Together, our findings demonstrate that irisin stimulates the release of a novel adipokine, CXCL1, via upregulation of NFκB pathway in human neck area derived differentiating adipocytes, which might play an important role in improving tissue vascularization.

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Expression of intracellular pattern recognition receptors in skeletal muscle

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Introduction: Skeletal muscle is one of the most versatile tissues in vertebrates. Besides playing a vital role in movement, breathing and acting as protein storage, it responds to a number of environmental factors, such as exercise and tissue damage. On the other side, skeletal muscle itself may act on other cells and tissues by producing various myokines (muscle-derived cytokines), such as IL-6 and IL-15. Many molecules contribute to the regulation of muscle function and myokine production. Like IFNγ was described as a negative regulator of muscle differentiation, furthermore, high levels of IFNγ is detected in various muscle-related inflammatory diseases.
Nod-like receptors (NLRs) are intracellular patters recognition receptors that sense specific patters of microorganisms, or potentially harmful and dangerous molecules. NLRs may regulate a wide scale of cellular functions including cytokine production, signal transduction pathways, cell proliferation or cell death. Importantly, the expression and function of NLRs in skeletal muscle has hardly been investigated yet.
Aims: To determine the basal and inducible expression of intracellular pattern recognition receptors in skeletal muscle. For this, we followed the NLR expression during myoblast-> myotube differentiation. We also examined the expression of pattern recognition receptors under the influence of IFN-γ in vitro and in vivo.
For the in vitro experiments, we used C2C12 immortalized skeletal cell line (myoblast or myotube). For our animal experiments we used C57BL/6J mouse. RNA isolation was carried out with TRIzol reagent, expression of PRRs was determined with RT-qPCR.
Results: During the 5 days differentiation, some of the receptors' expression was significantly upregulated (like NOD1), while were significantly downregulated (like NOD2). Following 6 and 24 hours IFNγ treatment, we detected significant upregulation in the case of NOD1 both in vitro and in vivo. To study the potential effect of IFNγ on NOD1- or NOD2- mediated cytokine expression, we used specific agonists of these receptors following IFNg treatment. Our results show that in myoblasts both, the NOD1 and NOD2 ligands significantly upregulated the expression of IL-6 in presence of IFNγ, while in mytobes only the NOD1 ligand had effect on the IL-6 expression.
Conclusion: NLRs are expressed in skeletal muscle, and their expression is changing during differentiation, also following IFNγ treatment. Modulation of NLR expression in skeletal muscle by cytokines, may contribute to muscle functions, including myokine production. However, the molecular mechanisms of the observed phenomena require further investigation.

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ZG16B, a rexinoid-regulated secreted factor increases mammary epithelial cell proliferation and migration

Máté Lengyel

Prevention of breast cancer formation with preventive medication is possible, but its effectiveness depends on finding optimal drug targets. Rexinoids are agonists of the retinoid X receptors and typically induce gene expression. In our current study we are focused on targets which are suppressed by rexinoid treatment and can be part of an antiproliferative and potentially chemopreventive mechanism. RNA sequencing was performed on immortalized normal mammary epithelial cells, and the suppression of ZG16B by bexarotene was confirmed with RT-qPCR. The suppression of ZG16B could be enhanced by synergistic combinations, such as bexarotene with paroxetine. ZG16B has a lower transcript level in healthy mammary tissue compared to primary and metastatic breast cancer.

Based on our hypothesis ZG16B can play a role in the regulation of proliferation and migration in breast cells, furthermore it can promote malignant transformation of breast cells. We also hypothesize, that the presence of ZG16B in the extracellular space can induce epithelial-mesenchymal transition (EMT) in breast epithelial cells.

To examine the biologic function of ZG16B, the cDNA of this gene was cloned into a pEGFP-C3 plasmid, and HEK293 cells were transfected with the construct. Expression of ZG16B was confirmed with RT-qPCR assay, Western-blot, and tandem mass-spectrometry analysis. Conditioned and concentrated supernatant from transfected HEK293 cells was used to treat in vitro normal (HMEC-hTert) and ‘malignant’ (MCF7, T47D, MDA-MB-231, HCC38, HCC1143, MDA-MB-468) breast cell cultures. Cell proliferation and migration (wound healing assay) was measured using automated microscopy and ImageJ based-image analysis. Activation of signaling pathways responsible for increased proliferation and migration (MAPK/Erk1/2, PI3K/Akt, Src) were examined by Western blots after ZG16B treatment. The effect of ZG16B on malignant transformation was measured with soft agar colony formation assay. EMT markers (CDH1, CDH2, VIM, FN1) were measured in T47D, MCF7 and MDA-MB-231 cells treated with ZG16B using RT-qPCR technique. The activation of NFkB and B-catenin proteins were monitored by measuring nuclear translocation of NF□B and □-catenin proteins with automated microscopy and image analysis.

Migration increased in all of the tested breast cell lines after ZG16B treatment, however it only affected proliferation of HMEC-hTert, MCF7 and T47D cells. Independently from hormone receptor expression status, Akt and Src kinases were activated after ZG16B treatment, but Erk1/2 phosphorylation could be only detected in HMEC-hTert. ZG16B has increased the number of spheroids in T47D and MDA-MB-231 soft agar colony formation assays.

Our results from the analysis of signaling pathways are consistent with the results obtained from proliferation and migration assays. Biological processes regulated by ZG16B can promote the formation and progression of breast cancer, therefore this protein can be a potential preventive and antitumor target. Finally, ZG16B may be raised as a biomarker for the prediction of the invasive behavior of early-stage breast cancers.

Palmitate inhibits efferocytosis by activating the mTORc1/RhoA/Rho kinase pathway

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Increasing evidence indicate that impaired clearance of apoptotic cells (efferocytosis) 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 the excess amount of FFA. In our experiments we are investigating whether exposure of macrophages to palmitate (a common dietary FFA) could affect the efferocytic 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 the expression levels of phagocytic receptors. Instead, exposure to palmitate resulted in increased mTORc1 activity leading to enhanced RhoA and Rho dependent kinase activation known to interfere with the phagocytosis of apoptotic cells. Accordingly inhibition of the AMPK-mTORc1 cellular energy-sensing signaling pathway or that of Rho kinase prevented the palmitate –induced inhibition of efferocytosis.
Our data indicate that metformin, an AMP kinase activator, used in the treatment of type 2 diabetes mellitus, might improve the symptoms of the disease also by preventing the palmitate-induced decrease in the efferocytic capacity of macrophages.

The role of the ABCB1 protein in cytotoxic lymphocytes

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Blocking the activity of the ABCB1 membrane transporter (also known as P-glycoprotein or P-gp and multidrug resistance protein 1 or MDR1) prevents the emigration of CD8+ lymphocytes from skin explants and their transmigration via human embryonic endothelial layers in models for lymphatic transmigration. It is known that ABCB1 related leukocyte alterations are manifested in several immunological diseases, such as multiple sclerosis, Crohn's disease, inflammatory bowel diseases, cancer, where cellular transmigration events may be implicated. However, there is limited information on the mechanism of how ABCB1 is involved in diapedesis via lymphatic endothelial cells. Our aim is to investigate the protein partners of the ABCB1 in human CD8+ lymphocytes and their role in transmigration. We determined the expression of CD8 and P-gp proteins in TALL-1 cells of a human cytotoxic cell line and in human primary cytotoxic T lymphocytes (CTLs) using monoclonal OKT8 and 15D3 antibodies. To increase the resting protein expression of the Pgp and CD8, we have modulated the protein expression of our cells by applying chromatin opener, hypoxia modeling, and immunomodulatory treatments known to induce P-gp. After different concentrations of Trichostatin A, Aza-deoxycytidine, cobalt chloride, dexamethasone, and IL-15, we have determined protein expression level kinetics using flow cytometry. The effects of the drugs mentioned above on the mRNA and protein levels will be analyzed using qPCR and western blots. The present measurements will be utilized in magnetic cell separation to select P-gp and

CD8 positive cells. Protein partners of the P-gp in CD8+ lymphocytes will be identified by mass spectrometry. So far, this study has revealed that both TALL-1 and CTLs express CD8+ protein makers with higher levels expressed in primary CTLs compared to TALL-1 cells, and that of dexamethasone upregulated the levels of P-gp in a time-dependent manner in primary CTLs.

Signal and cell type dependent role of cis regulatory elements in cytokine expression regulation and cells programming

Hamidreza Mianesaz

Dendritic cells (DCs) and macrophages are immune cells with a superior capacity to interpret internal and external stimuli. Depending on the activating signal such as different TLR ligands, the DCs and macrophages can activate different but overlapping sets of genes, and produce various sets of cytokines.
Using ChIP-seq and RNA-seq data previously generated in our group, a number of genes encoding cytokines, including IL12b, IL6, TNF, IL15 and CCL5, were selected for further analyses. These genes were investigated regarding to their regulatory landscapes. We defined a uniform approach to select the genes and their cis-regulatory elements. For all genes a list of promoter, distal and proximal putative enhancers and non-binding region(s) were selected and visualised using IGV tool. Based on the comparison, we concluded to proceed with IL12b as the gene of interest. We selected 6 enhancer regions as well as the promoter and negative control (non-regulatory) regions for editing the cis-regulatory element using CRISPR/Cas9 system. Simultaneously, we made efforts to setting up and optimization of our ChIP protocol by performing full ChIP-qPCR experiment.

An ultrarare manifestation of an X-linked recessive disorder: Duchenne muscular dystrophy in a female patient

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Duchenne Muscular Dystrophy (DMD) is an X-linked recessive dystrophin-associated neuromuscular disorder, boys are generally diagnosed between ages 3-5. Patients can be characterized with muscle weakness, gross motor delay and elevated serum creatinine kinase levels. The disease itself is caused by mutations in the DMD gene located on the Xp21.2-Xp21.1 chromosome. Since Duchenne Muscular Dystrophy is inherited X-linked recessively, it should only affect the hemizygous males. However, in rare cases, when unusual genetic mechanisms such as balanced translocations occur, heterozygous females can also have the same clinical

presentation and disease progress. Here we present an ultrarare manifestation of DMD in a female patient. A series of genetic examination revealed that she has an t(X;10)(p21.1;p12.1) translocation with one of the breakpoints in exon 54 of the DMD gene which is also accompanied by skewed X-inactivation and this resulted in the DMD phenotype of the girl. In conclusion, although in very rare cases, DMD can manifest in female patients as well. Performing whole genome sequencing in the first place would have been enough to determine the translocation and its breakpoints, significantly reducing the time required to establish a definitive molecular genetic diagnosis.

Section 5

ATRA and ATO combination has several beneficial effects in treatment of Acute Promyelocytic Leukaemia cells

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All-trans-retinoic acid (ATRA) therapy is one of the most frequently used therapy to treat acute promyelocyte leukaemia (APL) inducing terminal differentiation towards neutrophil granulocytes. Arsenic-trioxide (ATO) and ATRA and ATO combined treatments had been identified as another effective treatment in the late 90s' and then it was subsequently proved that this therapy can trigger both inductions of apoptosis and attenuation the inflammatory cytokine/chemokine production in vitro [Jambrovics et al., Cancer 2020]. Combined treatments can prolong the survival of APL patients in a dose-dependent manner by activation of the cellular signalling pathways leading to, among others, an enhanced reactive oxygen species (ROS) generation by the NADPH-oxidase system. ATO alone induces partial differentiation and apoptosis, leading to the remission in relapsed APL patients with the initiation of the degradation of the PML-RARα oncoprotein. ATRA and ATO combined treatments result in up and down-regulation of more than a thousand genes to generate functional neutrophil granulocytes. One of the most up-regulated genes in ATRA induced differentiation of NB4 APL cell line is the tissue transglutaminase (TG2). Silencing and 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., Haematologica 2018, Jambrovics et al., Cancer 2020]. To investigate the role of TG2 further, NB4 cell lines were treated with ATRA + ATO in two different combinations, where we found that without the TG2, NB4 cells were more sensitive to the arsenic-induced apoptosis. Additionally, we observed that the apoptosis induction by arsenic resulted in a degradation of the TG2 protein.

Study the role of miR-30 family members in the response of ovarian cells to high dose estrogen treatment

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Introduction: Estrogens are able to both trigger and inhibit cell proliferation in dose dependent manners. Cell proliferation is induced in nanomolar concentrations but higher doses inhibit cell proliferation and induce cell death instead. MicroRNAs are considered to play important role in estrogen response. Our aim was to characterize the function of miR-30 family members in the response of ovarian cells to high dose estradiol (E2) treatment.
Materials and methods: Two human epithelial ovarian cell lines were involved in the study: PEO1 (that express Estrogen Receptor α and β) and A2780 (that express ERβ only). The effect of high dose (1-100 μM) E2 was studied by determining cell proliferation, apoptosis, cellular lysis, mRNA and miR-30s expression.
Results: The basal expression of miR-30a-3p, miR-30a-5p, miR-30d-5p and miR-30e-5p proved to be higher in PEO1 than in A2780 that suggests their high biological relevance in ERα expressing ovarian cells. Furthermore, the expression of miR-30a-5p, miR-30d-5p and miR-30e-5p was induced in response to 50 μM E2 in PEO1. High dose E2 treatment reduced cell proliferation and induced cell death in ovarian cells that were accompanied with the induction of apoptosis (TP53) and autophagy (BAG3 and ATG2B) related genes. ERα-expressing PEO1 cells had higher tolerance to high dose E2 than A2780 that might be caused by the induction of ERα mediated estrogen response as it was suggested by the induction of GREB1 and CA12 genes. Bioinformatic analysis of miR-30s revealed that several targets are shared by miR-30a-5p, miR-30d-5p and miR-30e-5p in contrast to miR-30a-3p. Functional annotation of miR-30d-5p targets resulted in several pathways involved in the regulation of cell proliferation and cell death. The application of miR-30d-5p mimic reduced cell proliferation and decreased the tolerance of PEO1 cells to high dose E2.
Conclusions: According to our results miR-30a-5p, miR-30d-5p and miR-30e-5p might mediate the stress-response induced by high dose E2 in ovarian cells. MiR-30d-5p might be a promising therapeutic target in ovarian cancer.

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ARID1A mediates the anti-proliferative effects of bexarotene and carvedilol combination treatment in normal and transformed breast epithelial cells

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Keywords: synergy, rexinoids, breast cancer, SWI/SNF, ARID1A, IGF1, TGF-β

Drug synergy is the usage of a combination of agents to produce an effect that is greater than the sum of the effects generated through the administration of each drug individually. Retinoid X receptor (RXR) selective agonist, bexarotene (Bex), in combination with the non-selective beta blocker, carvedilol (Carv), showed synergistic anti-proliferative effects on normal epithelial breast cells (HMEC-hTert) and on the breast cancer cell line (MCF-7). In order to identify the molecular mechanism underlying these effects, we studied the proteomic profile and gene expression signatures of these cells upon the combination treatment of Bex +Carv. The results demonstrated that the protein levels of ARID1A, AT-rich interactive domain 1 A,

are induced upon the combination treatment in normal breast cells but not in cancer cells. We hypothesized that ARID1A modulated nucleosome organization upon Bex+Carv treatment to regulate expression of genes related to cell transformation and cancer development. To test our hypothesis, we performed chromatin immunoprecipitation followed by deep sequencing to identify ARID1A target regions under Bex+Carv treatment in normal and transformed cells. We also investigated the correlation between ARID1A enrichment and its target gene transcript and protein levels through RT-qPCR, Western-blotting and immunostaining assays. Moreover, we knocked down ARID1A using specific siRNA pool to study the impact of ARID1A on its target gene expression. The results showed that in MCF-7 cells ARID1A was enriched at regulatory elements related to insulin growth factor signaling pathway upon Bex+Carv treatment. These effects were associated with a downregulation in IGF-1R and IRS1 protein expression which was abolished upon ARID1A knockdown. The results suggest that one of the mechanism underlying the anti-proliferative effects of Bex+Carv treatment in MCF-7 is through the suppression of the IGF-1 signaling pathway activity which was orchestrated by ARID1A. In HMEC-hTert cells, ARID1A and BRG1 were recruited to regulatory elements related to transforming growth factor signaling pathway upon Bex+Carv. These changes were associated with an inhibition of the TGF-β pathway and its downstream activity reflected in the suppression of the EMT program, reflected in the levels of the epithelial markers fibronectin and N-cadherin. Overall, the results showed that bexarotene and carvedilol combination treatment behave differently in normal or transformed cells, showing anti-proliferative effects through targeting the IGF-1 or TGF-β signaling pathways. In both models, these effects were mediated by the actions of the tumor suppressor ARID1A.

Antidiabetics against cancer: metformin inhibits tumor growth by activating cohesin, interleukin 6, potentially affecting chromatin structure

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Metformin is a member of the biguanides (along with phenformin and buformin), which have a well-known antidiabetic effect. Though the other two members are banned due to their toxicity, metformin is still used nowadays as an approved antidiabetic drug. Researchers have also observed an inhibition effect of the biguanides on tumor growth, although the underlying mechanism remains unknown. The low toxicity metformin could be a novel drug candidate in cancer treatment if we understand how it affects cancerous cells. Therefore we treated MCF7 breast cancer cells with different concentrations of metformin, and measured the cell growth rate (compared to untreated control cells) with SRB assay and wound healing assay to get an accurate picture of the growth inhibition effect. We have extracted totalRNA and performed Next Generation Sequencing (NGS) to investigate the gene expression levels in each sample. We found that among the differentially expressed genes that are common between the treatments with different metformin concentrations, cohesin and interleukin 6 genes are consistently present. Other genes related to the cohesin complex, like the SMC or STAG family genes are also overexpressed, leading us to the conclusion that the cohesin complex, which is primarily responsible for the stability of chromatin loops, has an essential role in the antioncogenic effect of metformin.

Role of the transcription factor BACH1 in macrophage phenotype switch during tissue repair and regeneration

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After injury, circulating monocytes infiltrate the muscle and differentiate toward macrophages. At early time-points after the injury, the vast majority of infiltrating monocyte-derived macrophages present a pro-inflammatory phenotype, and later on there is a gradual transition from proinflammatory to anti-inflammatory phenotype. This process, named macrophage switch, is crucial for correct tissue repair; however, the chromatin-level mediators and regulatory events controlling this switch remain unknown. To delineate the timing of transcriptional changes, we used the cardiotoxin acute muscle injury model and we profiled chromatin accessibility and gene expression in sorted macrophage populations at different time-points of muscle regeneration. We identified a heme-binding transcriptional repressor, BACH1, as a novel regulator of this process. Bach1 knockout mice present an abnormal macrophage switch leading to impaired muscle regeneration and disrupting the expression of important genes involved in inflammation and tissue repair. To study how BACH1 affects the macrophage polarization during muscle repair we have performed Single Cell RNA-Seq experiments in WT and BACH1 LOF mice at different time points. These results show different clusters of macrophages depending on their gene expression pattern. Using WT / BACH1 LOF comparison will identify the magnitude of expression of critical inflammatory and repair-related genes.

Analysis of circulating miRNA profile in plasma samples of glioblastoma patients

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Background: Glioblastoma multiforme (GBM) is among the most aggressive cancers with a poor prognosis. Treatment options are limited, clinicians lack efficient prognostic and predictive markers. Circulating miRNAs – besides being important regulators of cancer development – may have potential as diagnostic biomarkers of GBM.
Methods: In this study, profiling of 798 human miRNAs was performed on blood plasma samples from 6 healthy individuals and 6 patients with GBM, using a NanoString nCounter Analysis System. To validate our results, five miRNAs (hsa-miR-433-3p, hsa-miR-362-3p, hsa-miR-195-5p, hsa-miR-133a-3p and hsa-miR-29a-3p) were randomly chosen for RT-qPCR detection.

Results: 53 miRNAs were significantly differentially expressed in plasma samples of GBM patients when data were filtered for FC□1 and FDR□0,1. Target genes of the top 39 differentially expressed miRNAs were identified, and we carried out functional annotation and pathway enrichment analysis of target genes via GO and KEGG-based tools. General and cortex-specific protein–protein interaction networks were constructed from the target genes of top miRNAs to assess their functional connections.
Conclusions: We demonstrated that plasma microRNA profiles are promising diagnostic and prognostic molecular biomarkers that may find an actual application in the clinical practice of GBM, although more studies are needed to validate our results.

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Section 6

High Level of Thiamine is Required for Efficient Thermogenesis in Human Neck Adipocytes

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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. Brown/beige adipocytes are characterized by the expression of uncoupling protein-1 (UCP1) that enables them to dissipate energy as heat instead of generating ATP. 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 that thiamine transporter 2 (ThTr2), encoded by SLC19A3 gene, was upregulated in DN adipocytes.
We found that inhibition of ThTr2 by its specific inhibitor fedratinib during thermogenic activation of SC and DN adipocytes by cAMP (which mimics in vivo adrenergic stimulation) decreased UCP1-dependent proton leak respiration and hampered cAMP-dependent upregulation of browning marker genes (UCP1, CKMT2, and PGC1a) as well as mitochondrial complex subunits. In the absence of thiamine in the culture fluid, cAMP-induced elevation of proton leak respiration and the expression of browning markers were abrogated, but both were restored by thiamine in a concentration dependent manner.
To further understand the mechanism of action of thiamine, which is converted to the biochemically active compound thiamine pyrophosphate (TPP) in cells, we designed a seahorse-based respiration assay that reflects the activity of one of the TPP dependent enzyme, pyruvate dehydrogenase (PDH) in cell membrane-permeabilized cells. We found that addition of TPP could significantly increase PDH activity dependent proton leak respiration of adipocytes.

In conclusion, our ex vivo study revealed the importance of ThTr2 and the availability of thiamine during thermogenic activation in human SC and DN adipocytes. Thiamine increases heat generation by upregulating the expression of UCP1 and other browning marker genes, and by directly acting on TPP-dependent enzymes that are not fully saturated by TPP.

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Mitophagy is upregulated during beige to white transition of human subcutaneous abdominal adipocytes

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Brown and beige adipocytes have multilocular lipid droplets, increased mitochondrial mass, express high level of uncoupling protein (UCP) 1, and promote energy expenditure. In rodents, when the stimulus of browning subsides, parkin-dependent mitophagy is activated and dormant beige adipocytes persist. In humans, however, the molecular events during beige to white transition were not studied in detail. We attempted to differentiate the adipocytes in a human model for a long period of time, because our previous and present results show a sharp, time-dependent separation of the white and beige adipocytes. Previously, our research group carried out 28 days experiment on Simpson-Golabi-Behmel syndrome cell line, in which the beige differentiation resulted in an increased brown, beige marker gene and protein expressions, as well as oxygen consumption, which were partially maintained after the beige to white transition. In this study, human primary subcutaneous abdominal preadipocytes were differentiated to beige for 14 days, then either the beige culture conditions were applied for additional 14 days or it was replaced by a white differentiation medium. As a negative control, white adipocytes were differentiated by their specific cocktail for 28 days. Peroxisome proliferator-activated receptor γ-driven beige differentiation resulted in increased mitochondrial biogenesis, UCP1 expression, fragmentation, and respiration as compared to white. Morphology, UCP1-content, mitochondrial fragmentation, and basal respiration of the adipocytes that underwent through beige to white transition, along with the induction of mitophagy, were similar to white adipocytes. However, converted beige adipocytes had a stronger responsiveness to dibutyril-cAMP, which mimics adrenergic stimulus, than the white ones. Removal of mitochondria involved both parkin-dependent and independent pathways. Our results verify in a human ex vivo model that the beige to white transition is linked to mitophagy. Prevention of the cell autonomous transition along with mitochondrial clearance can be a novel approach to keep energy dissipation high to alleviate obesity and type 2 diabetes mellitus.

Long-Term Thiamine Treatment Promotes Thermogenic Competency of Human Adipocytes

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Brown adipose tissue (BAT) dissipates energy as heat mainly via the mitochondrial uncoupling protein-1 (UCP-1) activity. The activation of BAT was postulated as a potential therapeutic target to ameliorate obesity. Therefore, finding novel agents that can stimulate the differentiation and recruitment of brown or beige thermogenic adipocytes in humans are important subjects for investigation. In this study, we aimed to investigate the effect of continuous thiamine (vitamin B1) treatment during adipocyte differentiation on the expression of thermogenic marker genes and proteins.
We treated primary human adipogenic progenitors that were cultivated from subcutaneous (SC) and deep neck (DN) adipose tissue and SGBS preadipocytes with excess amount of thiamine during their 14 days differentiation program. Long-term thiamine administration did not influence the mRNA and protein expression of either thiamine transporter (ThTr) 1 or 2 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 that there was an increasing trend in the expression of mitochondrial complex subunits 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 both 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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The effect of alpha-ketoglutarate on phosphate and hypoxia-induced calcification of vascular smooth muscle cells

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Introduction: Recent studies revealed that hypoxia contributes to vascular calcification via the activation of hypoxia-inducible factor 1 (HIF-1) pathway. The alpha-ketoglutarate-dependent prolyl hydroxylase domain enzymes regulate the stability of HIF-1α in response to oxygen availability. As a rate-limiting substrate, alpha-ketoglutarate regulates the activity of prolyl hydroxylases, therefore we addressed the inhibitory effect of alpha-ketoglutarate on vascular smooth muscle cell calcification under hypoxic condition.
Methods: We induced calcification of human aortic smooth muscle cells (HAoSMCs) and mice aortic rings with elevated inorganic phosphate (Pi, 2 mmol/L) and hypoxia (1% O2) in the presence or absence of alpha-ketoglutarate (1-10 mmol/L). Protein expressions of HIF-1α, and glucose transporter-1 were evaluated by Western blot. Extracellular matrix (ECM) mineralization was assessed by Alizarin Red staining, calcium measurement and osteocalcin ELISA.

Results: Alpha-ketoglutarate dose-dependently inhibited Pi+hypoxia-induced Ca accumulation in the ECM of HAoSMCs. Osteocalcin level of Pi+hypoxia-treated HAoSMCs was largely attenuated by alpha-ketolglutarate (14.9 vs. 0.01 ng/mL). Alpha-ketoglutarate inhibited Pi+hypoxia-induced elevation of HIF-1α and Glut-1 expressions. Alpha-ketoglutarate attenuated Pi+hypoxia-induced calcification of mice aortic rings (0.2 vs. 0.03 mg Ca/mg protein).
Conclusions: Alpha-ketoglutarate decreases Pi+hypoxia-induced osteochondrogenic transdifferentiation and ECM mineralization of HAoSMCs in vitro and mice aorta rings ex vivo. Further studies are necessary to investigate the effect of alpha-ketoglutarate on vascular calcification in vivo.

Dexamethasone and high glucose induce osteogenic differentiation of human lens epithelial cells

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Introduction: Cataract, or opacification in the lens, is the global leading cause of blindness and visual loss. In many developing countries cataracts are common among adolescent and cataract is considered as an aging disease. Interestingly, cataract is frequently associated with atopic disorders such as diabetes, or certain medications, such as the corticosteroid dexamethasone. It has been suggested that lens calcification is a cell-mediated process, however the mechanism is yet to be elucidated. In certain aspects lens calcification is similar to vascular calcification; both of them are aging diseases and accompanied by hydroxyapatite deposition in the soft tissues. Vascular calcification is mediated by a phenotype switch of vascular smooth muscle cells (VSMCs) into osteoblast-like cells. Previously our group showed that similarly to VSMCs, human lens epithelial cells (HuLECs) can undergo osteochondrogenic differentiation in response to osteogenic stimulation. Our aim is to investigate the effect of high glucose and dexamethasone on osteochondrogenic differentiation of HuLECs.
Methods: HuLECs were purchased from ScienCell Research Laboratories and cultured as suggested by the company. Calcification of HuLECs were induced by osteogenic medium (OM) supplemented with phosphate (2.5 mmol/L) and calcium (0.3 mmol/L) in low-glucose and high-glucose DMEM. Dexamethasone was used at a concentration range of 50-200 nmol/L. Extracellular matrix (ECM) calcification was determined by Alizarin red staining and ECM calcium determination. Protein expressions were determined by western blot and ELISA, while mRNA levels were assessed by quantitative real-time PCR.
Results: As revealed by Alizarin red staining and ECM calcium measurements high glucose intensified OM-induced ECM calcification of HuLECs. High glucose induced the expressions of the osteogenic transcription factor Runx2 and the chondrogenic transcription factor Sox9. Dexamethasone dose-dependently intensified OM-induced ECM calcification of HuLECs assessed by Alizarin red staining and ECM calcium measurements.
Conclusion: Both high glucose and Dexamethasone intensify OM-induced calcification of HuLECs. Further experiments are needed to investigate the involvement of this cellular mechanism in cataract formation in diabetes and corticosteroid treatment.

Activation of Nrf2/HO-1 axis by heme attenuates calcification of human lens epithelial cells

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Introduction: According to the Global Eye Health Statistics, at least 25 million eyes develop decreased visual acuity due to cataract formation annually. Currently, cataract is the leading cause of blindness worldwide. The etiology of cataract is diverse, however, aging is the most common risk factor for cataract formation. There are a lot of similarities between vascular calcification and cataract; both of them are aging diseases, associated with excess production of reactive oxygen species (ROS) and accompanied by hydroxyapatite deposition in the vessels and the lens respectively. Vascular calcification is a cell-mediated process, caused by a phenotype switch of vascular smooth muscle cells (VSMCs) into osteoblast-like cells. Similarly to VSMCs, human lens epithelial cells (HuLECs) can undergo osteogenic transition and gain osteoblast-like features upon osteogenic stimulation. Nuclear factor erythroid 2-related factor 2 (Nrf2) regulates the expression of a variety of antioxidant genes, and plays a protective role in vascular calcification. Our aim was to investigate the effect of Nrf2 upregulation on osteogenic differentiation of HuLECs.
Methods: HuLECs were purchased from ScienCell Research Laboratories and cultured as suggested by the company. Calcification of HuLECs were induced by osteogenic medium (OM) supplemented with phosphate (2.5 mmol/L) and calcium (0.3 mmol/L). Nrf2 induction was achieved by heme treatment (1-50 μmol/L). Extracellular matrix (ECM) calcification was determined by Alizarin red staining and ECM calcium determination. Protein expressions were determined by western blot and ELISA, while mRNA levels were assessed by real-time PCR.
Results: Heme dose-dependently upregulated Nrf2 protein expression and inhibited OM-induced ECM calcification in HuLECs. Heme-mediated Nrf2 activation was accompanied by upregulation of heme oxygenase-1 (HO-1). Heme lost its potential to inhibit calcification in the presence of ML385 and tin-protoporphyrin, pharmacological inhibitors of Nrf2 and HO-1 enzyme activity, respectively. End-products of HO-1-mediated heme degradation (bilirubin, CO and Fe(II)) inhibited OM-induced HuLECs calcification to different extents.
Conclusion: We concluded that heme-mediated activation of the Nrf2/HO-1 axis protects against OM-induced calcification in HuLECs with the involvement of heme-degradation products.

Section 7

DIA spectral libraries for salivary biomarkers to identify oral malignancies

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The 6th highest prevalence malignancy is oral squamous cell carcinoma (OSCC). Besides the genetic determination, the main etiological risk factors are smoking and alcohol consumption. Early diagnosis is critical, because the 5-year survival rate is only 50%. OLK (oral leukoplakia) is one of OSCC's precancerous states, it is a white plaque which cannot be scrapped. Other type of premalignant states is OLP (oral lichen planus), which affects the mucous membrane of the oral cavity in an immune mediated way. The chance of the transformation of these diseases into malignancy is approximately 25% (OLK) and 5% (OLP), respectively. Nowadays, the diagnosis of OSCC is based on histological examination. Unfortunately, there are no screening tests available, but the examination of salivary biomarkers seems to be a promising tool. Our aim was to identify specific salivary proteins with mass spectrometry analysis to identify OLK, OLP and OSCC.
Saliva samples were collected from patients with OLK, OLP or OSCC and age- and sex-matched controls. Bradford and BCA methods were used to determine the protein concentration then the samples were digested with trypsin. Finally, using data-independent acquisition (DIA) mass spectrometry the digested samples were analyzed.
As a result of the analysis, DIA spectral libraries were generated for OLK, OLP and OSCC groups and the libraries were used for screening to find potential salivary biomarkers
The proteomic analysis of salivary samples became possible with the developed DIA spectral library and it found to be useful for screening biomarkers for OSCC and its premalignant stages.

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Development and characterisation of a transglutaminase 2 knocked down endothelial cell model

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Keywords: transglutaminase 2, endothelial cell, cell death, cell adhesion

Transglutaminase 2 (TG2) is a unique multifunctional protein. In addition to the best-characterised Ca2+-dependent transglutaminase activity, it also has GTPase, ATPase, protein disulfide isomerase, and protein kinase activities. It participates in many biological processes (e.g., signalling, phagocytosis, apoptosis) and may be associated with immunological, tumour, and neurodegenerative diseases. Endothelial cells covering the surface of blood vessels are particularly important in the maintenance of hemostasis, and human umbilical cord vein derived endothelial cells (HUVEC) express high levels of TG2 protein.
We aimed to investigate the effect of TG2 silencing on HUVEC cells to reveal the importance of TG2 in human endothelial cells.
For inducible silencing of TG2 expression, a synthetic oligonucleotide with a sequence homologous to the UTR region of TG2 mRNA was cloned into the pLKO-Tet-Puro vector. HUVEC cells, previously immortalised by telomerase overexpression, were transduced by

replication-incompetent lentiviruses and selected using puromycin. The silencing of TG2 was induced with doxycycline.
The decrease in TG2 expression was demonstrated by RT-qPCR and Western blot. Altered adhesion abilities and an increase in the proportion of apoptotic cells were observed in the TG2 silenced cells. This further confirms that TG2 plays a role in cell adhesion and survival. TG2 protein level was restored by viral intake of an inducible, triple FLAG-tag fused transgene form, which partially compensated for the altered processes.
In order to more precisely explore the biological functions of TG2 in HUVEC cells and understand their molecular mechanism, we plan to identify TG2 interaction partners and substrates in the developed cellular model.

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Investigation of the conformational epitope of mouse monoclonal antibody 885 recognizing a celiac-related surface in transglutaminase 2

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Keywords: celiac disease, transglutaminase 2, antibody interference

Objective: Transglutaminase 2 (TG2) is the main autoantigen in celiac disease (CeD). TG2 has several biologically relevant conformational epitopes, including those recognised by CeD autoantibodies. We investigated the binding epitope of mAb885, a non-commercial antibody (developed and owned by Thermo Fisher Scientific, ImmunoDiagnostics, Sweden) able to interfere with CeD antibody binding to TG2 (Simon-Vecsei, PNAS 2012).
Methods: Competition with purified natural and cloned CeD patient-derived antibodies was investigated. Pointmutants, truncated TG2 and chimeric human-mouse TG2 proteins were produced and tested with mAb885 in ELISA. Interference with other members of the TG family was assessed by sequence analysis, structural studies and recombinant TG enzimes. Biological effects on cultured cells were studied by resazurin-dependent adhesion assays.
Results: mAb885 selectively interferes with the binding of CeD antibodies to celiac epitope 2. Binding of mAb885 is highly conformational and recognises only structurally intact epitopes. Both the N-terminal and core domains of human TG2 have relevant anchor points for its binding, but mutation of the main anchor point of epitope 2 (R19) does not decrease its binding. mAb885 has no interaction with other members of transglutaminase family and does not have the same biological effects in cell culture as CeD antibodies.
Conclusions: The investigated antibody has a partially overlapping binding site with celiac antibodies but no similar biological effects and may thus be useful for future biological therapies. Further, mAb885 is suitable to assess the structural integrity and amount of epitope 2 in various diagnostic ELISA tests for CeD.

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Comparative analysis of transcriptomic changes induced by xenoestrogens in ovarian cells

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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). The aim of our study was to compare the transcriptomic changes induced by physiological and xenoestrogens in ovarian cells.
Materials and methods: PEO1 human epithelial ovarian cell line was treated with estradiol (10 nM), zearalenone (10 nM) and bisphenol A (100 nM). Total RNA was isolated 8h later. MRNA sequencing was performed on Illumina NextSeq 500. Validation of expression data was made by qPCR.
Results: According to our results estrogen treatments induced remarkable alterations in gene expression: the expression of 1740, 1896 and 860 genes changed significantly ($p<0.05$) in response to estradiol, zearalenone and bisphenol A, respectively. Among these, 304, 283 and 62 genes were up-regulated ($log2FC>1$); 288, 255 and 44 genes were down-regulated ($log2FC<-1$) markedly. Overlaps in gene expression alterations was also determined. 319 genes were co-regulated in response to estradiol and zearalenone and 81 genes were up or down regulated in response to all the three estrogens tested. According to functional enrichment analysis the induced genes highly enriched in cell division (e.g.: DNA replication, cell cycle, chromosome organization) and non-coding RNA processing (e.g.: NcRNA metabolic process, NcRNA processing) related pathways. Down-regulated genes were enriched in epithelial differentiation (e.g.: epithelial cell differentiation, epithelium development) and cell adhesion (e.g.: biological adhesion, cell adhesion) related pathways. 10 genes were chosen for validation the expression results by qPCR. Pierson correlation coefficient proved to be $r=0.91$, $r=0.96$ and $r=0.95$ between gene expression data obtained by mRNA sequencing and qPCR in response to estradiol, zearalenone and bisphenol A, respectively.
Conclusion: Zearalenone and estradiol induce comparable changes in gene expression. Bisphenol A induces less genes but these mostly overlap with those that are induced by estradiol. These results are in good agreement with our previous phenotypic studies where estrogens induced cell proliferation, migration and changes in microRNA expression.
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Optimization of the myeloid blood differentiation from pluripotent embryonic stem cells via embryoid body formation

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To investigate the potential of embryonic stem cells (ESCs) for patient-centered approach of immunotherapy, we need to know in detail the process in which direct the ESCs to differentiate. To accelerate the ex vivo differentiation of ESCs toward mesodermal and blood cells we can use OP9 feeder-cells. We previously observed that with this OP9 co-culture method the ratio

of the obtained myeloid cell population was rather low (5-20%) and variable. ESCs can also be differentiated without feeder cells via embryoid body (EB) formation. EBs can be formed by culturing the cells in hanging drops in two days; after that orbital shaker can be used to further grow the cell aggregates for a few days. Thereafter the EBs can be disaggregated and the obtained mesodermal cells can further be cultured in the presence of GM-CSF to stimulate the myeloid blood cell development. In this study we compared the blood cell differentiation capacity of the EBs making them with or without the hanging drop method. In addition, we tested the impact of GM-CSF during the last phase of the differentiation. To evaluate the blood cell formation we assessed the c-Kit, CD45 and CD34 cell surface expression with flow cytometry testing two different murine ESC lines (R1 and ZX1). Our results revealed that EBs can be created without hanging drop formation and these EB derived cells can efficiently convert to CD45+ blood cells. Importantly, we obtained a high percentage of blood cell development after 12 days of cell differentiation. Moreover we observed a more efficient blood cell formation if we grow the EBs for 6 days instead of 5 days. In addition, we observed a better differentiation with the R1 ESC line compared with the ZX1 cells. Finally, we found that in the absence of GM-CSF, much less CD45+ blood cells were generated. These findings suggest that the EB based protocol is more suitable for the ESC derived myeloid blood cell differentiation. In the future we plan to carry out more experiments testing additional cytokines. Moreover, we intend to characterize some transgenic cell lines using this optimized ESC differentiation protocol.

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IMPAIRED NLRP3 PATHWAY ACTIVITY IN VIRUS EXPOSED HUMAN PLASMACYTOID DENDRITIC CELLS

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Introduction: As professional type I interferon (IFN) producing cells, plasmacytoid dendritic cells (pDC) show a high type I IFN signaling pathway activity in response to viruses, thus they are one of the most effective participants of the antiviral immune responses. However, the NLRP3 pathway, which determines immune responses to bacterial infections, is still unexplored in these cells. Thus, we aimed at investigating the interaction of type I IFN and IL-1β pathways in human pDCs.
Methods: In our experiments, pDCs were exposed to various species of live bacteria, fungi and viruses then the NLRP3 dependent IL-1β response was examined by ELISA, western blot and flow cytometry. To explore the interaction between the antiviral and antibacterial responses of pDCs, their bacteria-induced IL-1β production was also determined in the presence of RNA and DNA viruses, which are potent type I IFN inducers.
Results: We observed that pathogenic bacteria such as E. coli have a higher capacity to induce NLRP3 activation in pDCs, in contrast to commensal bacteria or viruses. However, the E. coli induced IL-1β production was diminished in the presence of RNA (VSV) or DNA (HSV-1) viruses that is probably due to the inhibitory effects of virus-induced type I IFNs on the NLRP3-dependent IL-1β pathway. Furthermore, we directly confirmed that the presence of IFN-α in the cell culture media significantly decreased the TLR-, and nigericin-induced IL-1β production of pDCs suggesting that type I IFNs inhibit NLRP3 inflammasome activity in pDCs.

Conclusion: Our results show that antagonistic effects can be observed between the antiviral type I IFN and the antibacterial IL-1β pathways in human pDCs. These findings indicate that the IL-1β-mediated responses of pDCs may prevail in inflammatory conditions, where the type I IFN pathway is not dominant.

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Functional characterization of two PNLIP variants identified in patients with pancreatic lipase deficiency

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Pancreatic lipase (PNLIP) deficiency is a rare digestive disorder characterized by fat malabsorption, greasy stool and vitamin deficiency. The disease is typically caused by loss-of-function mutations affecting both alleles of the PNLIP gene. A recent clinical study reported twin brothers with the symptoms of PNLIP deficiency. Genetic testing indicated that the patients carried two compound heterozygous mutations in the PNLIP gene, which changed Trp102 to a stop signal (W102X) and Arg188 to Cys (R188C). Our aim was to study the mechanisms by which these PNLIP mutations cause the disease.
PNLIP mutations were generated by site directed mutagenesis of the PNLIP-10His-pcDNA3.1(-) mammalian expression vector. Secretion of PNLIP variants was studied by analyzing the conditioned media or cell lysates of transiently transfected HEK 293T cells with protein gel electrophoresis followed by Coomassie staining or immunoblotting, respectively. Total RNA was purified from cell lysates. Intracellular mRNA levels of ER stress markers immunoglobulin binding protein (BiP) and C/EBP homologous protein (CHOP) were monitored with quantitative RT-PCR. The levels of X-box binding protein 1 (XBP1) mRNA splicing was followed by RT-PCR and agarose gel electrophoresis.
We found that in contrast to the wild-type PNLIP protein, which was readily secreted by the cells, variants W102X and R188C were not observed in the conditioned media. Analysis of the cell lysates indicated that the R188C PNLIP variant was expressed but not secreted probably due to protein misfolding, whereas the W102X PNLIP variant protein was not present in the cells likely due to nonsense-mediated decay. Consistent with this notion, only the misfolding R188C PNLIP variant caused ER stress indicated by increased expression of BiP and CHOP and slightly elevated levels of XBP1 splicing.
Our results demonstrated that pancreatic lipase deficiency of the patients is due to defective secretion of PNLIP variants.

Purification of Schistosoma japonicum GST with metal ion affinity chromatography

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The glutathione-S-transferase of Schistosoma japonicum (sjGST) is one of the most widely applied fusion tags in the recombinant protein technology. The fusion of the target protein with GST may increase its solubility, helps its proper folding, and enables its purification with

glutathione-affinity chromatography. It was found previously that the sjGST protein can bind a divalent ion, and this ion-binding ability can be improved by the modification of the protein. The E26H mutant was found to show improved ion-binding ability as compared to the wild-type sjGST, and can be purified using nickel-chelate affinity chromatography, as well, but the mutant protein was not characterized in details.
In this work we aimed the comparative analysis of the wild-type and E26H mutant sjGST proteins, with a special emphasis on their interactions with glutathione and metal ion affinity surfaces. The sjGST was modified using site-directed mutagenesis, the wild-type and E26H mutant proteins were expressed in BL21(DE3) bacterial cells. Glutathione- and nickel-nitrilotriacetic acid (Ni-NTA) magnetic beads were used to immobilize the proteins, the interactions with the affinity surfaces were investigated by washing the beads with buffers containing the eluents (glutathione or imidazole, respectively) at increasing concentrations. The eluted fractions were analyzed by SDS-PAGE, followed by densitometric analysis of the gels.
We successfully introduced the E26H mutation to sjGST, and studied the wild-type and mutant proteins in vitro. The E26H mutation had no effect on glutathione-binding while remarkably improved the binding to Ni-NTA and cobalt-NTA surfaces. The in vitro results were correlated with the ion-binding ablities predicted in silico. Neither glutathione nor imidazole eluents impaired the binding of sjGST proteins to Ni-NTA and glutathione-affinity surfaces, respectively. The results revealed that the E26H sjGST can be efficiently purified with Ni-NTA, the optimization of the conditions for a two affinity step-containing purification protocol is in progress.

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Cell-free nucleic acids and their application in diagnosing glioblastoma multiforme

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Glioblastoma is the most common and aggressive primary brain tumour (phase IV), with rapid growth, inevitable recurrence, and median survival of 12–15 months. Diagnosis is usually made with imaging techniques and biopsy samples or surgical resection. Unfortunately tissue biopsy is a risky procedure and only represents a small area of the tumour. Liquid biopsy is a promising source of biomarkers such as DNA, RNA and proteins. This technique is more repeatable procedure than tissue biopsy, has a much lower risk and can be used for monitoring treatments. MicroRNAs, long non-coding RNAs and circRNAs are also useful biomarkers, as they have highly tissue specific expression patterns and depends on their physiological function and tumour status, by playing key role in tumour progression, proliferation, angiogenesis and even therapy resistance via dysregulating important signalling pathways.
Our main goal is to find cell free nucleic acids which may be promising biomarkers in diagnosis of glioblastoma multiforme using non-invasive method.

Interactions among lncRNAs, miRNAs and circRNAs were predicted by bioinformatics tools. 40 samples (23 tissue sample and 17 plasma samples) were collected. RNA was isolated from tissue, plasma and exosomes, and then reverse transcribed. Some microRNAs, long non-coding RNAs and circRNAs were selected and then studied by real time PCR.
Based on our pilot study, alterations in gene expression profile were observed between control and GBM groups, but no significant differences were studied among long non-coding RNAs, microRNAs and circRNAs, however more samples are needed to study.
In conclusion, some selected non-coding RNAs might be possible biomarker candidates, however further investigations are required to reveal the underlying molecular mechanisms among non-coding RNAs. Our future knowledge about these non-coding RNAs might support the early diagnosis and personalized treatment of glioblastoma.

Invasive aspergillosis specific miRNA signatures in oncohematology patients

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Keywords: invasive aspergillosis, hematological malignancies, small RNA sequencing, microRNAs, blood biomarkers

Invasive fungal infections (IFIs) have emerged over the 40 years as an important cause of human disease. Aspergillus fumigatus is the most prevalent and is largely responsible for the increased incidence of invasive aspergillosis (IA) in the immunocompromised patient population. IA is responsible for one of the most devastating lung infections in terms of morbidity and mortality. Many factors have likely contributed to the emergence of IA, including neutropenia, HIV epidemic, prolonged use of corticosteroids and immunosuppressive agents, organ transplantations, nosocomial exposure and hematological malignancies. However, standardized, case-finding methodologies of IA are lacking, so there is a need for new and alternative diagnostic strategies. The application of blood biomarkers, like nucleic acid targets has become a research trend. Nowadays, the connection between extracellular microRNA levels and several pathological processes, including different infections are increasingly recognized. MicroRNAs are class of small (19-24 nt), noncoding RNAs that can regulate gene expression post-transcriptionally. Numerous studies reported the potential of free circulating microRNAs as disease biomarkers in diagnosis and a reliable tool for future use.
The prime aim of this study was to identify IA specific circulating miRNA signatures in the whole blood samples of oncohematology (OH) patients that could serve as good biomarkers for the prompt diagnosis of invasive aspergillosis. Therefore, we performed an analysis of high-throughput small RNA sequencing data obtained from 26 HO patients and 24 healthy controls. In silico bioinformatics analyses revealed 36 differentially expressed miRNAs between IA-infected and non-IA OH patients. Among these, we found 8 miRNAs (hsa-miR-191–5p, hsa-miR-106b-5p, hsa-miR-16–2-3p, hsa-miR-26a-5p, hsa-miR-15a-5p, hsa-miR-20a-5p, hsa-

miR-106a-5p and hsa-miR-17–5p) with high specificity and sensitivity to discriminate the IA-infected and non-IA OH patients, which were also validated by qRT-PCR measurements.
This pilot study is the first effort to understand the levels of circulating miRNAs to identify stable, disease-specific diagnostic markers. Finding possible non-invasive biomarkers at early stages of disease progression may reduce the shortcomings of current tests and improve patient outcomes. In our study, by the application of next generation sequencing, we managed to find 8 potential miRNA biomarkers in the early diagnosis of IA in oncohematology patient groups. These IA-specific miRNAs have the potential to serve as good biomarkers for disease diagnosis and may also lead to a better understanding of IA pathogenesis.

Zymosan-induced immune response in alternatively polarized macrophages

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Macrophages are key players in the immune barrier against pathogen infections. These innate immune cells are highly heterogenic and plastic. Their phenotype and function are primarily depending on their molecular microenvironment and origin. Based on the microenvironmental signals-induced polarization, two phenotypic end-point can be distinguished, the IFNγ-induced classical (M1) and the IL-4-mediated alternative (M2) macrophage polarization. In certain diseases like allergic asthma and fibrosis, macrophages can be polarized in an M2 way, which influences the phenotypic characteristics and immune response against the pathogens such as fungi. The pathogen-associated molecular pattern recognizing receptors, like Dectin-1 and the Tlr2, have an essential role in the recognition of invading pathogens and the antifungal immune response.
We aimed to study the expression of Dectin-1 and Tlr2 in IL-4-polarized murine macrophages, as well as the inflammatory response of M2 macrophages following Saccharomyces cerevisiae cell wall component Zymosan treatment.
Murine bone marrow cells were differentiated into macrophages using an M-CSF-containing L929 cell supernatant. Then these cells were primed by IL-4 and treated with Zymosan to perceive their antifungal immune response. The expression of "Zymosan recognizing receptors" (Clec7a, Tlr2), alternative macrophage polarization marker genes (Arg1, Fizz1, Ym1), pro-inflammatory cytokines (TNFα, IL-1β, IL-6), and chemokines (Ccl2, Ccl22, Ccl17) was examined by RT-qPCR method. In addition, pro-inflammatory (TNFα, IL-6) and the anti-inflammatory cytokine (IL-10) secretion were studied by ELISA method. Finally, the phagocytosis of the fungal particles was followed up by Texas-Red dye conjugated Zymosan under the fluorescent microscope.
We observed that IL-4-primed macrophages showed an elevated Dectin-1 (Clec7a) and decreased Tlr2 mRNA expression. Moreover, the IL-4 repressed the Zymosan induced Tlr2 expression, while the Dectin-1 (Clec7a) relative mRNA level was further induced after the dual

treatment. Phagocytosis of the Zymosan was more efficient after IL-4 treatment. Zymosan could reduce the IL-4-enhanced Arg1 mRNA level, while the other M2 polarization marker genes, including Fizz1 and Ym1 mRNA levels, remained almost unchanged. Although the Zymosan-induced TNFα mRNA expression level was augmented by IL-4 priming, the secreted cytokine level proved to be higher following IL-4 polarization. Both IL-6 mRNA and secreted protein levels were synergistically increased by IL-4 priming and Zymosan activation. The anti-inflammatory IL-10 protein secretion was the highest in IL-4-primed and Zymosan-exposed macrophages, but the mRNA expression level did not show any differences between the alternatively and non-polarized macrophages. The Ccl2, Ccl22, and Ccl17 chemokine mRNA expression levels were synergistically increased in the alternatively polarized macrophages following Zymosan activation.
According to our observations, the IL-4-induced alternative polarization and the Zymosan-activated signaling pathways regulate the macrophage's antifungal immune response complexly.

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Section 8

What is beyond protein identification? Opportunities for targeted and data-independent protein analysis.

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Protein identification is one of the most common procedure in proteomics. The classical proteomics strategy involves shotgun data-dependent acquisition (DDA) where the top 10-20 intensive ions are selected for fragmentation and based on the recorded fragment ion spectra, the protein identification can be done. This procedure has an internal bias due to the selection of the most intensive ions and in this case we could identify the abundant proteins in the sam-ple. However, biological and clinical research studies often focus on those proteins which have relatively lower abundance in the biological systems, and with classical DDA methods they are not necessarily selected for fragmentation.
As an alternative of the shotgun analyses targeted mass spectrometry acquisition can offer the specific and selective analysis of the proteins of interest. Our high resolution Orbitrap Fusion mass spectrometer offers Parallel Reaction Monitoring (PRM) acquisition method where only pre-selected peptides are selected for fragmentation. The selection of the peptides is the critical step of the PRM experiment, unique protein specific peptides called proteotypic peptides should be found and selected for the acquisition. Besides the accurate identification PRM offers quantitative data as well.
Another alternative of DDA experiments is the data-independent acquisition (DIA) which is an unbiased mass spectrometry technique of the new bench-top high-resolution mass spectrometers that can eliminate the major drawbacks of the classical DDA-based approaches. DIA combines the sequential isolation of a large precursor window with full product ion spec-trum

thus, the analysis of every precursor ion in the samples is possible without any kind of selection. One of the major benefit of DIA experiments is that the information about the whole proteome can be stored in a digitalized form and can be made publicly available.
The presentation will contain examples for the utility of PRM and DIA analyses in biological and medical sciences.

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Analysis of the cellular proteo-transcriptomic changes following HIV-1/2 transduction in HEK-293T cells

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Lentivirus-based vectors derived from human immunodeficiency viruses type 1 and 2 (HIV-1 and 2) are widely used tools in research and may also be utilized in clinical settings. While both viruses share a similar genomic and organizational structure, there are significant differences in their replication cycle and infectivity. Lentiviruses rely on host cell machinery for replication, and compared to HIV-1, interactions between the cellular components and HIV-2 is less well studied. Our aim 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. Analysis of the proteomic data showed that in the first 2 hours of transduction, 7 proteins were significantly downregulated 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. At the 8-hour time-point, only the aldolase fructose bisphosphatase 1 (ALDOC1) was found to be downregulated by both viruses, with no other significant proteomic changes 12 hours post-transduction. 26 hours after transduction, both viruses altered the cellular level of 41 proteins which are involved in signaling, mRNA processing and translational activities. Among the affected proteins were the cleavage and polyadenylation specificity factor subunit 6 (CPSF6) and heterogeneous nuclear ribonucleoprotein A2 (hnRNPA2), which were shown to be important in the replication of HIV. Moreover, differential regulation of proteins such as Ran binding protein (RanBP1) and Parkinsonism associated deglycase 7 (PARK7) was observed for HIV-1 and HIV-2. Both viruses managed to significantly alter the transcriptome of the host cell in all of the time points compared to those of the controls. Transduction with HIV-1 changed the expression of a wide variety of genes coding for transcriptional factors, metabolic enzymes and proteins involved in the cell cycle in the different time points of transduction. Meanwhile, HIV-2 significantly increased the expression of protein coding genes involved in the formation of the extracellular matrix and inhibitors of viral proteins. Our data showed significant differences in the remodeling of cellular proteome and transcriptome induced by HIV-1 and HIV-2-based pseudovirions, and help us understand the pathomechanism of infection, as well as enrich our knowledge about the use of HIV-based lentiviral vectors.

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High throughput wine vinegar metabolite profiling by UPLC-Orbitrap Fusion Tribrid MS and programmatic annotation: A methodological approach.

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Background: Wine vinegar is an acidic liquid prepared from grape-must by subsequent alcoholic fermentation with Saccharomyces cerevisiae strains followed by acetous fermentation with Acetobacter species. Various types of fruit vinegar have served as seasoning-, preservative-, or treatment agents ever since ancient civilizations. Among the Hungarian grape juice fermentation products, mostly wines, especially from the Tokaj region, have been the subjects of analytical chemical investigations, including our comprehensive metabolomic analyses. To our knowledge, there are no such studies that have been performed to determine the overall metabolite composition of vinegar produced from Hungarian wines.
Materials and methods: We analyzed four different Furmint wine vinegars from the Tokaj region using metabolomics approaches followed by computational evaluation of the obtained data. The metabolite profiling was carried out by reverse-phase, ultra-performance liquid chromatography coupled to Orbitrap Fusion Tribrid mass spectrometry (RP-UPLC-Orbitrap Fusion Tribrid MS) with data-dependent acquisition. We processed the resulting chromatograms and corresponding MS1 and MS2 spectral data with the Compound Discoverer 3.1 spectral evaluation software. The metabolite identification relied on the mzCloud mass spectral-, ChemSpider compound-, and Arita Flavonoid structural databases. To annotate the identified compounds, we queried the PubChem Compound, BioAssay, and PubMed databases via their application programming interfaces (APIs).
Results: Metabolites from diverse chemical classes, including saccharides, amino acids, organic acids, phenolic compounds, chalcones, stilbenoids and lignans were identified. In addition to the water-soluble components, fatty acids, sterols, and terpenes were also detected. Several of the individual compounds were associated with cell line and animal studies in the literature that reported anticancer and anti-inflammatory effects.
Conclusions: Fragmentation at the MS2 level allowed the exact identification of only a few flavonoids and lignans. However, the characteristic fragment ions of isobaric flavonoids, such as flavones, isoflavones, flavonols, flavanones, flavanonols and flavan-3-ols, and lignan-derivatives were detected in multiple peaks. Repeating the analyses at the MS3 level, and the use of commercial standard molecules may significantly increase the number of identified higher molecular weight secondary plant metabolites. The determination of more biologically active components in wine vinegars may inspire their use as precursors of new functional food products.

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Network analysis of atheroma and complicated lesions in human atherosclerosis

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An atheroma is basically an accumulation of macrophages, lipids, and debris in the arterial wall that can be caused by factors such as hypertension, smoking, diabetes, obesity, or insulin resistance. In order to obtain more information about the protein composition of atheroma's, the major objective of our project was to perform an extensive network analysis of the data obtained from the study of the different types of samples.
Proteins were extracted from atheromatous lesions, complicated lesions, and healthy arteries. Proteins were cleaved with trypsin and identified from MS/MS spectra using MaxQuant and EncyclopeDIA. Network generation and analysis were performed using String-db and Cytoscape, respectively. The ClueGo plugin was used for pathway analysis, while gene-gene interactions were determined using CluePedia. CytoHubba was used to determine the top hub proteins in the network.
We have examined that most of the oxidation events occur in the complicated lesions. Oxidations were mainly, but not exclusively, related to haemoglobin. Functional analysis revealed extensive overlaps between networks.

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Proteomics and metabolomics profiling of serum from patients with obesity or type 2 diabetes

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Obesity has become a serious health problem in many developing countries in recent decades. Obesity over a long period of time can lead to persistently high glucose concentrations in the blood and is considered as one of the major risk factors for the development of type 2 diabetes. Patients with type 2 diabetes can suffer from numerous complications, generally macrovascular and microvascular diseases. Therefore, we aimed to profile proteins and metabolites, including amino acids and biogenic amines, in serum samples from patients with type 2 diabetes and patients with obesity and healthy people.

A total of 85 subjects were recruited for this study, 26 patients with type 2 diabetes, 31 obese subjects, and 28 healthy volunteers. 100 µl of serum sample from each participant was derivatized with AccQ-Tag Ultra derivatization kit (Waters). The concentration of 20 proteinogenic amino acids, 3 non-proteinogenic amino acids, and 10 biogenic amines were measured using UPLC (Waters) coupled to 5500 QTRAP (Sciex) mass spectrometer. The proteomes of the serum were analyzed using Easy nLC 1200-Orbitrap Fusion (Thermo-Scientific) system following trypsin and Lys C digestion.
Serum concentrations of Ser, Gly, Asp, Glu, Thr, and Cit were significantly decreased, while serum Cys, Val, Ile, and Leu levels were increased in both disease groups compared to the control group. The concentrations of serum Cys and Cit were statistically significantly higher, whereas serum levels of Asp and Ile were lower in the obese group compared to the type 2 diabetes group. Only serum ethanolamine was significantly decreased in the disease groups compared with the control group. Methylamine, ethylamine, putrescine, and serotonin were detected but not quantified. The protein lists and peptide sequences acquired from the proteomics examination of serum sample were examined along with the data acquired from the examination of small molecules. Our results may provide a better understanding of obesity and type 2 diabetes.

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Establishing the mutational spectrum of Hungarian patients with familial hypercholesterolemia

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Abstract: Familial hypercholesterolemia (FH) is one of the most common autosomal dominantly inherited diseases affecting the cholesterol metabolism which, in the absence of treatment, leads to the development of cardiovascular complications. The disease is still underdiagnosed, even though an early diagnosis would be of great importance for the patient to receive proper treatment and to prevent further complications. No studies are available describing the genetic background of Hungarian FH patients. In this work, we present the clinical and molecular data of 44 unrelated individuals with suspected FH. Sequencing of five FH-causing genes (LDLR, APOB, PCSK9, LDLRAP1 and STAP1) has been performed by next-generation sequencing (NGS). In cases where a copy number variation (CNV) has been detected by NGS, confirmation by multiplex liga-tion-dependent probe amplification (MLPA) has also been performed. We identified 47 causal or potentially causal (including variants of uncertain significance) LDLR and APOB variants in 44 index patients. The most common variant in the APOB gene was the c.10580G>A p.(Arg3527Gln) missense mutation, this being

in accordance with literature data. Several missense mutations in the LDLR gene were detected in more than one index patient. LDLR mutations in the Hungarian population largely overlap with mutations detected in neighbouring countries.