7th Molecular Cell and Immune Biology Winter Symposium

organized by the
Molecular, Cellular and Immune Biology Doctoral School, University of Debrecen, VÉD-ELEM TÁMOP Program and UD-GenoMed

Galyatető 7-10 January, 2014

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ÚJ SZÉCHENYI TERV

7th Molecular Cell and Immune Biology Winter Symposium 2014

Programme:

7 January

14:30 Opening: László Fésüs

14:40-16:20 Section 1 Chair: Zoltán Balajthy

14:40-14:55 András Mádi:
Summary on Doctoral School activities

14:55-15:20 László Fésüs:
How science and scientific publications may go wrong

Introductory lectures

15:20-15:50 Zsolt Bacsó:
MEDICINE Nobel Prize 2013

15:50- 16:20 István Szatmári:
New discoveries on cellular reprogramming

16:20-17:00 Coffee and cake

17:00-18:30 Section 2 Chair: Bálint Bálint

Regular talks

17:00-17:15 Ixchelt Cuaranta Monroy:
Genome wide analysis during adipocyte differentiation from mouse embryonic stem cells

17:15-17:30 Endre Kristóf:
Second generation antipsychotic (SGA) drugs modify the differentiation program of human adipocytes inducing "browning" markers

17:30-17:45 Péter Brázda:
SPIMming around the nucleus: a nuclear mobility map of transcription factors in live cells

17:45-18:00 Attila Pap:
Generation of mice lacking PPARg in the hematopoietic system by embryonic liver cell transplantation

18:00-18:15 Erika Takács:
Comparison the expression of seventeen dendritic cell specific transcription factors in embryonic stem cells- and bone marrow-derived dendritic cells

18:15-18:30 Zoltán Simándi:
PRMT1 and PRMT8 are context-dependent regulators of retinoid-induced neural differentiation

18:30-20:00 Dinner

20:00-21:30 Section 3 Chair: Lóránt Székvölgyi

Introductory lecture

20:00-20:30 Mónika Fuxreiter:
CHEMISTRY Nobel Prize: All atom modelling of proteins

Regular talks

20:30-20:45 Rashmi Sharma:
Nanny model for intrinsically disordered proteins

20:45-21:00 Gergely Nagy:
A novel method to predict regulatory regions based on histone mark landscapes

21:00-21:15 Júlia Koller:
Regulatory SNPs with possible clinical significance: Genome-wide ChIP-seq based searching and analysis of transcription factor binding sites in human and mouse

21:15-21:30 Mahdi Mohamed:
A modular system for HIV-2 protease analysis

8 January

7:00-8:30 Breakfast

8:30-10:35 Section 4 Chair: Attila Bácsi

Introductory lectures

8:30-8:55 Lóránt Székvölgyi:
C or not C with Hi-C: the 3D organization principles of eukaryote genomes

8:55-9:20 Zsuzsanna Nagy:
Mapping the IL4 induced STAT6 transcriptome and cistrome in primary human macrophage like cells

Regular talks

9:20-9:35 András Szántó:
R loops: who R they?

9:35-9:50 György Fenyőfalvi:
Quantifying DNA superhelicity and chromatin loop length in eukaryotic cell nuclei

9:50-10:05 István Németh:
Silenced expression of TG2 attenuates the LPS induced proinflammatory response of NB4 neutrophil granulocytes through TLR2, TLR4 – NF-kB signaling pathway

10:05-10:20 Esther Bokhobza:
DCNP1 (Dendritic cell associated nuclear protein-1), a potential player in the NFkB pathway

10:20-10:35 Zsolt Czimmerer:
The IL-4/STAT6 signalling pathway regulates evolutionarily conserved miRNA signature in macrophages

10:35-16:00 Free programme

15:30-16:00 Coffee and cake

16:00-18:30 Section 5 **Chair: István Szatmári**

Introductory lectures

16:00-16:30 Zoltán Prohászka (Semmelweis University):
Role of innate mechanisms in the pathogenesis of thrombotic thrombocytopenic purpura

16:30-17:00 Attila Bácsi:
Lymphocyte subtypes involved in allergic inflammation

Regular talks

17:00-17:15 Krisztián Horváth:
The role of 8-oxoG in pollen-induced allergic inflammation

17:15-17:30 Krisztina Köröskényi:
How does adenosine inhibit LPS-induced pro-inflammatory cytokine formation in macrophages?

17:30-17:45 Kitti Linda Pázmándi:
Effect of native and oxidatively modified exogenous mitochondrial DNA on the functions of human plasmacytoid dendritic cells

17:45-18:00 Anna Pallai:
mTNF alpha signalling inhibits-LPS-induced proinflammatory cytokine formation by upregulating TGF-betą in macrophages

18:00-18:15 Zsófia Agod:
SLAMF5 is a regulator of CD40L-induced responses in plasmacytoid dendritic cells

18:15-18:30 Boglárka Tóth:
Focal adhesion proteins in coeliac and control HUVECs

18:30-20:00 Dinner

20:00-21:35 Section 6 **Chair: Goran Petrovski**

Introductory lecture

20:00-20:20 Bálint Bálint:
Dissecting the Genetic and Epigenetic Components of Gene Expression Regulation in the Context of the 1000 Genomes Project

Regular talks

20:20-20:35 Gergely Ivády:
Molecular diagnostics of cystic fibrosis: the mutational spectrum in Eastern Hungary and the development of a NGS-based method.

20:35-20:50 Ágnes Fekete:
Molecular epidemiological studies in age-related macular degeneration

20:50-21:05 Melinda Paholcsek:
An alternative way in the diagnosis of aspergillosis; a possible example of translational medicine

21:05-21:20 Péter Lábiscsák:
Identification of saliva biomarkers of oral squamous cell carcinoma in Hungarian population using targeted proteomics method and multiplexed immunobead-based assay

21:20-21:35 Beáta Bartáné Tóth:
Looking behind the morphological changes of primary HUVEC-cell-lines

9 January

7:00-8:30 Breakfast

8:30-10:40 Section 7 Chair: Zsuzsa Nagy

Introductory lecture

8:30-8:55 Goran Petrovski:
Functional and molecular characterization of ex vivo cultured epiretinal membrane cells from human proliferative diabetic retinopathy

Regular talks

8:55-9:10 Ildikó Bacskai:
CD55 and CD59 complement regulatory proteins participate in dendritic cell activation by lipopolysaccharide

9:10-9:25 Bence Dániel:
Cistromic features of liganded RXR's action in macrophages define a distinct, partly hidden angiogenic enhancer network

9:25-9:40 Szabolcs Tarapcsák:
Comparative analysis of P-glycoprotein (ABCB1) expressing and non-expressing JIMT-1 breast cancer cell subpopulations

9:40-9:55 Gábor Szalóki:
Dissection of the catalytic cycle of Pgp using Walker A mutants

9:55-10:10 Arunima Chatterjee:
The impact of colonic epithelial cell derived stimuli on myeloid cells and T-lymphocytes

10:10-10:25 Pál Krisztián Bene:
Inflammatory responses regulated by human monocyte-derived dendritic cells in the gut

10:25-10:40 Bea Kiss:
Some new aspects in the signalling of negative selection in thymocytes

10:40-16:00 Free programme

15:30-16:00 Coffee and cake

16:00-17:40 Section 8 **Chair: Gábor Zahuczky**

Introductory lectures

16:00-16:20 Éva Csősz:
Go targetted - the power of targeted proteomics in the study of antimicrobial and innmunmodulatory peptides

16:20-16:40 Róbert Király:
Separation of transglutaminase 2 isopeptidase and transamidase activities

Regular talks

16:40-16:55 Kiruphagaran Thangaraju:
Possible role of transglutaminase 2 activities and variants in aggresome formation

16:55-17:10 Gergő Kalló:
Quantification of human alpha and beta defensins in colonic epithelial cells by targeted MRM-based proteomics

17:10- 17:25 Katalin Nagy:
Retroviral-like aspartic protease ASPRV1

17:25-17:40 Lívia Gazda:
Study on Ty1 and Ty3 retrotransposon proteases

17:40-18:40 Section 9 (Poster section) **Chair: Róbert Király**

Dóra Bojcsuk:
Meta-analysis of the ERα CISTROME in various human cell lines

Eszter Boldizsár:
Growth of the A2058 melanoma cell line is regulated by the adaptor SH3PXD2B/HOFI

Pál Botó:
Modulation of early dendritic cell development with lineage determining transcription factors

Erik Czipa:
Evolutionary conservation of transcription factor binding sites in human and mouse

Edina Erdős:
Chromatin studies for mapping of the Estrogen Receptor α binding sites in the human MCF7 cell line

István Fedor:
LXR signaling is inducible in glioma cell lines and is a putative regulator of cell migratory capacity

Gábor Fidler:
Diagnostic accuracy of PCR compared to galactomannan in serum samples of invasive systematic aspergillosis

Dorottya Hajdú:
Effect of antipsychotics on the fagocytic ability of monocyte-macrophage
László Halász:
Chromosomal R-loops mediate spatial chromosomal interactions
Attila Horváth:
Modelling RXR-regulated gene expression dynamics in bone-marrow derived macrophages using steady-state and nascent RNA levels
Kajal Kanchan:
Structural determination of human Transglutaminase 2 in presence of calcium
Máté Kiss:
Activation of retinoid X receptor induces a proangiogenic macrophage phenotype in vitro
Zsuzsanna Kolostyák:
Enhanced adipoyte differentiation of mouse embryonic stem cells
Gergő Kovács:
Studies on the human NOD-like receptor family CARD domain containing 5 (NLRC5) protein
Arnold Markovics:
Analysis of gene copy number variations in malignant hematologic diseases
Anett Mázló:
Inhibitory role of NLRX1 in human dendritic cells immunosupressed by MSCI
Lilla Ozgyin:
Setting up epigenetic studies on 12 B-limphoblastoid cell lines of the 1000 Genomes Project
Márk Pilling:
Carbohydrate surface markers profiling of ex vivo cultured epiretinal membrane cells from human proliferative diabetic retinopathy
Zsolt Raduly:
Arc, memory, fuziness: What's in common?
Tibor Sághy:
Compounds enhancing intracellular cAMP levels increase the expression of transglutaminase 2 in mouse thymocytes
Zsanett Sári:
Activation of LXR signalling modulates neural differentiation of mouse embryonic stem cells
Márta Tóth:
The effects of the wild type *Lactobacillus casei* BL23 and its peptidoglycan-hydrolase mutants on human monocyte-derived dendritic cell functions
Trang Bảo Khanh:
Genome-wide analysis of Hepatocyte nuclear factor-1 & -4 binding sites in correlation with maturity-onset diabetes of the young
Máté Végső:
Purification and in vitro characterization of a mutant capsid protein of HIV-1

18:40-20:00 Dinner

20:00-21:40 Section 10 **Chair: Zsolt Bacsó**

Introductory lectures

20:00-20:20 Tamás Varga:
Origin and function of macrophages in tissue regeneration

20:20-20:40 Zsolt Sarang:
Macrophages produce a novel retinoid to enhance their phagocytic capacity during long term phagocytosis of apoptotic cells

Regular talks

20:40-20:55 Anitta Sárvári:
Characterization of inflammatory reactions of human macrophages to differentiating adipocytes

20:55-21:10 Éva Garabuczi:
Involvement of the LXR receptor in the glucocorticoid-induced enhancement of apoptotic cell phagocytosis

21:10-21:25 Marika Tóth:
Clearance of dying RPE cells by professional and non-professional phagocytes as in vitro model for age-related macular degeneration (AMD)

21:25-21:40 Gergely Joós:
Are adenosine A3 receptors required for proper phagocytosis of apoptotic cells in vivo?

10 January

7:00-8:30 Breakfast

8:30-10:25 Section 11 **Chair: Éva Csősz**

Introductory lecture

8:30-8:50 Zsolt Keresztessy:
Genome engineering technology developments for research and therapy

8:50-9:10 Gábor Zahuczky:
Collaborative RTD with/in UD-GenoMed. Abundant Tear Protein Depletion Kit for biomarker discovery

Regular talks

9:10-9:25 Péter Nánási:
Laser scanning cytometric analysis of DNA end-labelling reactions on nuclear halosamples

9:25-9:40 László Imre:
Nucleosome-DNA affinity is highly sensitive to certain H3 modifications and to superhelical twist

9:40-9:55 Elvan Ergülen:
Functional relevance of DNAJ A1 as a novel interacting partner of human transglutaminase 2

9:55-10:10 Ferenc Tóth:
Characterization of the cleavage site-mutant capsid proteins of HIV-1

10:10-10:25 Minh Doan:
Seeing the cell face, knowing the cell fate

10:25 Concluding remarks: Éva Rajnavölgyi (chair)
József Tőzsér
Zsuzsa Szondy
Mónika Fuxreiter

ABSTRACTS

SECTION 1

New discoveries on cellular reprogramming

István Szatmári
Stem cell Research Laboratory, Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen, Hungary

There were several interesting discoveries on cell reprogramming at 2013. American scientists announced that they had cloned human embryos with somatic cell nuclear transfer and used them to establish embryonic stem cell lines (therapeutic cloning). Chinese researchers reprogrammed mouse somatic cells into pluripotent stem cells using small-molecule compounds without any transcription factor introduction (pure chemical iPS cells). Finally two papers described a highly efficient iPS cell generation from somatic cells. The aim of this presentation is to briefly introduce these new findings.

Grant support: TÁMOP-4.2.2.A-11/1/KONV-2012-0023

SECTION 2

Genome-wide analysis of adipocyte differentiation from mouse embryonic stem cells

Ixchelt Cuaranta-Monroy[1], Zoltan Simandi[1], Attila Horvath[1,2],Gergely Nagy[1,2],Endre Barta[1,2]and Laszlo Nagy[1,2]
1 Nuclear Hormone Receptor Laboratory, Department of Biochemistry and Molecular Biology, Research Center for Molecular Medicine, University of Debrecen, Medical and Health Science Center.
2 MTA-DE "Lendulet" Immunogenomics Research Group, University of Debrecen, Debrecen, Hungary, H-4012

Both the excess and the lack/atrophy of adipose tissue are associated with altered metabolism and lead to major diseases. Therefore, several models have been set up for dissection of fat cell differentiation. We used an improved adipocyte differentiation protocol for embryonic mouse embryonic stem cells (ESCs) that allows us to get insight in the early phases and late stages of adipogenesis due to its high efficiency and monolayer output. We carried out genome wide analysis during adipocyte differentiation (RNA-seq and ChIP-seq): samples for RNA seq were taken at different time points along the differentiation (day 0, 3, 4, 7, 8, 15, 21 and 27) and ChIP-seq samples for histone modification markers and transcription factors RXR were taken at day 0, 15 and 27. Hierarchical clustering in the RNA-seq data combined with H3K27ac, an active histone mark,ChIP-seq results in a dynamic change in gene expression during adipocyte differentiation recognizing clusters for general gene expression analysis and transcription factor expression. This systematic analysis allows us to identify known and novel regulators of adipogenesis such as HIF/AP-1, TEAD and MADS factors. Further dissection of this data will allow us to get insight in adipocyte differentiation and potential new targets for therapy in obesity.

Work in the Nagy laboratory is supported by a grant from the Hungarian Scientific Research Fund (OTKA K100196), and TÁMOP-4.2.2.A-11/1/KONV-2012-0023 VÉD-ELEM implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund.

Second generation antipsychotic (SGA) drugs modify the differentiation program of human adipocytes inducing "browning" markers

Endre Kristóf[1], Doan Xuan Minh[2], Anitta Sárvári[1], Zoltán Balajthy[1], Zsolt Bacsó[2], László Fésüs[1]
[1]*Apoptosis, Genomics and Stem Cell Research Group, Department of Biochemistry and Molecular Biology, University of Debrecen*
[2]*Department of Biophysics and Cell Biology, University of Debrecen*

Although brown adipose tissue (BAT) can be found only in small amounts in the human body after infancy, recent studies revealed that it has a major importance in regulating the energy balance of the entire body. Its oxidative metabolism contributes to energy expenditure during cold exposure and diet or physical exercise induced thermogenesis triggering the recruitment of "brown adipocyte-like" or "beige" cells interspersed in subcutaneous white adipose tissue depots in a process called "browning". A very strong negative correlation has been found between obesity and BAT amount in humans. Targeting the currently known and unknown regulatory systems of "browning" might open up better strategies to specifically stimulate BAT in obese individuals to aid weight reduction.
We observed that in vitro antipsychotic drug treatment at clinically administered concentrations reprogrammed the gene expression pattern of differentiating human adipocytes, surprisingly leading to overexpression of the major BAT marker gene, UCP1. Our aim was to clarify whether a commonly used second generation antipsychotic drug (SGA) was able to induce a browning program on differentiating human adipocytes.
Human primary preadipocytes obtained from herniotomy were differentiated into white or brown adipocytes with or without long or short-term SGA treatment. Expression of white, brown and general adipocyte markers were determined by RTQ-PCR, immunoblotting or immunocytochemistry and changes in morphology (size and number of lipid droplets, expression of Ucp1 and Cidea in situ) were visualized and quantified by Laser Scanning Cytometry.
SGA administration resulted in a significant overexpression of several brown adipocyte marker genes (UCP1, ELOVL3, CIDEA, CYC1, PGC1A) and Ucp1 protein.SGA treated cells had more and smaller lipid droplets, more mitochondrial DNA and expressed more Ucp1 and Cidea in situ than the in vitro differentiated white adipocytes.We have concluded thatSGA treatment at clinically administered concentrations is able to induce a browning program in differentiating human white adipocytes in vitro.

This research was realized in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 „National Excellence Program – Elaborating and operating an inland student and researcher personal support system convergence program" The project was subsidized by the European Union and co-financed by the European Social Fund."

SPIMming around the nucleus: a nuclear mobility map of transcription factors in live cells

Peter Brazda[1,2], Jan Krieger[3], Jörg Langowski[3], György Vámosi[4] and Laszlo Nagy[1,2]
[1]*Department of Biochemistry and Molecular Biology,*
[2]*MTA-DE "Lendület" Immunogenomics Research Group,*
[3]*German Cancer Research Center, Division Biophysics of Macromolecules, Heidelberg, Germany,*
[4]*Department of Biophysics and Cell Biology, Research Center for Molecular Medicine, University of Debrecen, Medical and Health Science Center, Debrecen, Hungary*

Retinoid X Receptor (RXR) is a promiscuous nuclear receptor forming heterodimers with several other receptors, which activate different sets of genes. The working model of nuclear receptor action is mainly based on biochemical approaches do not take into account the dynamic behavior of a transcription factor. By applying FRAP and fluorescence correlation spectroscopy (FCS), techniques with different temporal and spatial resolution, a highly dynamic behavior could be uncovered, which is best described by a two-state model of receptor mobility. In the unliganded state most RXRs belonged to the fast population, leaving ~15% for the slow, chromatin bound fraction. Upon agonist treatment, this ratio increased to ~43% as a result of an immediate and reversible redistribution.

Single plane illumination microscopy based FCS (SPIM-FCS) is a new method for imaging FCS in 3D samples. A nuclear mobility map recorded by this technique shows that the ligand-induced transition occurs throughout the nucleus. In my presentation I will present a brief introduction into SPIM-FCS focusing on the microscopy setup, the data evaluation and interpretation.

A recent extension of the technique is the SPIM-FCCS, which allows measuring molecular interactions in an imaging mode, in live cells. With our focus on nuclear receptor function we are aiming to investigate the protein-protein interactions and complex formation of RXR and its partners during activation.

This work was supported by grants from the Hungarian Scientific Research Fund (OTKA K100196 to L. N., K77600 and K103965 to G. V.), the Deutscher Akademischer Austausch Dienst (MÖB/21-1/2013 to G. V.), TÁMOP-4.2.2.A-11/1/KONV-2012-0023 VÉD-ELEM and by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.4.A/ 2-11/1-2012-0001 'National Excellence Program'.

Generation of mice lacking PPARg in the hematopoietic system by embryonic liver cell transplantation

Attila Pap[1], Eva Pintye[2], Edit Hathy[1], Tamas Varga[1] and Laszlo Nagy[1, 3]
1, Nuclear Hormone Receptor Research Laboratory, Department of Biochemistry and Molecular Biology
2, Department of Radiotherapy
3, MTA-DE "Lendulet" Immunogenomics Research Group

The fatty acid activated transcription factor PPARg has been linked to adipocyte differentiation, macrophage and dendritic cell function and also to diseases such as diabetes, atherosclerosis and chronic inflammation. However assigning cellular functions to the receptor in various cell types has been hampered by the lack of appropriate genetic models in

which PPARg is completely eliminated from a given cell type or an entire animal. In this study we aim at the development and characterization of a complete PPARg null mouse line generated by crossing PPARg floxed and epiblast specific Sox2Cre transgenic mouse lines. The PPARg-KO mice have phenotypic abnormalities and for this reasons the newborn KO mice are more sensitive for the environmental conditions, and many of them die in the first few days. To generate appropriate amount of mice for investigation the role of PPARg in hematopoesis and immune cell differentiation, we set up embryonic liver cell transplantation method which could replace the bone marrow transplantation.
We lethally irradiated the BoyJ congenic mice (recipients), which strain have a CD45.1 type cell surface marker on the white blood cells, and transplanted with PPARg-KO donor embryonic liver cells. The PPARg-KO mice carry the CD45.2 type of this marker, which allow us to monitoring the repopulation efficiency 8 weeks after transplantation by flow cytometry. The method works well, we could generate chimeras which the 98-100% of hematopoietic cells are repopulated by PPARg-KO donor cells. Now we use this model to investigate the modification of gene transcription in macrophages lacking PPARg.

Work in the Nagy laboratory is supported by a grant from the Hungarian Scientific Research Fund (OTKA K100196), and TÁMOP-4.2.2.A-11/1/KONV-2012-0023 VÉD-ELEM implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund.

Comparison the expression of seventeen dendritic cell specific transcription factors in embryonic stem cells- and bone marrow-derived dendritic cells

Erika Takács
Stem Cell Research Laboratory, Department of Biochemistry and Molecular Biology - University of Debrecen, Debrecen, Hungary

Dendritic cells (DCs) are antigen-presenting cells which play a critical role in the regulation of the adaptive immune response. DCs can be generated from various precursors including embryonic stem (ES) cells (ES-DC). However it was demonstrated that ES-DCs had an impaired T cell activation capacity to compare bone marrow (BM)-derived dendritic cells. Our hypothesis is that some key factors are missing from the ES cell-derived DCs, and this contributes for the impaired T cell stimulation capacity of these cells.
To investigate this possibility we have systematically compared the gene expression profiles of the ES cell- and BM-derived DCs.We have assessed the mRNA levels of seventeen DC specific transcription factors (Irf8, Relb, Egr1, Egr2, Sfpi1/PU.1, Irf2, Mafb, Tcf4, Maf, Ikzf1, Batf3, Spib, Runx3, Irf4, Id2, Zbtb46 and Bcl6) with real-time PCR from ES-and BM-DCs. Interestingly three transcription factors showed a significantly lower expression in ES-DCs in two independent cell differentiation systems. These results suggest that professional antigen presenting cells from different sources have distinct gene expression patterns. The observed differences in transcription factor expression between ES- and BM-DCs might contribute for the impaired immunogenicity of the ES-derived immune cells.

Grant support: TÁMOP-4.2.2.A-11/1/KONV-2012-0023 and TÁMOP-4.2.2/B-10/1-2010-0024

PRMT1 and PRMT8 are context-dependent regulators of retinoid-induced neural differentiation

Zoltan Simandi[1], Szilárd Póliska[2], István Juhász[3], László Imre[4], Balazs Dezso[5], Gábor Szabó[4], Álmos Klekner[6], Bálint Bálint L.[2] and Laszlo Nagy[1,7]
[1]*Nuclear Hormone Receptor Research Group, Department of Biochemistry and Molecular Biology;* [2]*Department of Dermatology;* [3]*Department of Biochemistry and Molecular Biology, Center for Clinical Genomics and Personalized Medicine;* [4]*Department of Biophysics and Cell Biology;* [5]*Department of Pathology;* [6]*Department of Neurosurgery;* [7]*MTA-DE "Lendület" Immunogenomics Research Group University of Debrecen, Medical and Health Science Center*

Retinoids are morphogens and have been implicated in cell fate commitment of embryonic stem cells (ESCs) to neurons. Their effects are mediated by RAR and RXR nuclear receptors. However, transcriptional co-factors required for cell and gene-specific retinoid signaling are not known. Here we show that Protein aRginine Methyl Transferase (PRMT) 1 and 8 have key roles in determining retinoid regulated gene expression and cellular specification in a multistage neuronal differentiation of murine ESCs. PRMT1 acts as a selective modulator, providing the cells with a mechanism to reduce the potency of retinoid signals on Hox genes. PRMT8 is a retinoid receptor target gene itself and acts as a cell type specific transcriptional co-activator of retinoid signaling at later stages of differentiation. Lack of either of them leads to reduced nuclear arginine methylation and dysregulated neuronal gene expression. We propose that PRMT1 and PRMT8 serve as a rheostat of retinoid signaling to determine neuronal cell specification, and might also be relevant in the development of neoplastic as well as degenerative diseases of the brain.

Zoltan Simandi is junior research fellow of the University of Debrecen, Medical and Health Science Center and recipient of the Jedlik Ányos fellowship. This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence Program'. Work in the Nagy laboratory is supported by a grant from the Hungarian Scientific Research Fund (OTKA K100196), TÁMOP-4.2.2.A-11/1/KONV-2012-0023 VÉD-ELEM and TÁMOP-4.2.2.C-11/1/KONV-2012-0010 HPC-NVL implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund. Balint L. Balint is Szodoray fellow of the University of Debrecen Medical and Health Science Center and recipient of the Magyary Zoltan fellowship supported by the TÁMOP 4.2.4.A/2-11-1-2012-0001 grant, implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund.

SECTION 3

A novel method to predict regulatory regions based on histone mark landscapes

Gergely Nagy[1], Bence Dániel[1], Dávid Jónás[1], László Nagy[1,2] and Endre Barta[1,2]
[1]*University of Debrecen, Medical and Health Science Center, Department of Biochemistry and Molecular Biology, Center for Clinical Genomics and Personalized Medicine,* [2]*MTA-DE "Lendulet" Immunogenomics Research Group, University of Debrecen, Debrecen, Hungary, H-4012*

Macrophages as phagocytes and professional antigen presenting cells play critical role in both innate and adaptive immunity. Main transcription factors acting during their differentiation and function are known, but the behavior and co-operation of these factors still

remained unexplored. We introduce a new approach to map nucleosome-depleted regions using exclusively active enhancer and core promoter marking histone modification ChIP-seq data. We could detect approximately 56,000 potential active enhancers/promoters showing different lengths and histone modification shapes. Beside the highly enriched PU.1 and C/EBP sites, we could also detect binding sites for RUNX and AP-1, as well as for the MiT (MITF-TFE) family and MEF2 proteins. The PU.1 and C/EBP transcription factors are known for transforming cells into macrophages. The other transcription factors found in this study can play a role in macrophages as well, since it is known that the MiT family proteins are responsible for phagocytic activity and the MEF2 proteins specify monocytic differentiation over the granulocyte direction. Our results imply that this method can provide novel information about transcription factor organization at enhancers and core promoters as well as about the histone modifications surrounding regulatory regions in any immune or other cell types.

Gergely Nagy is junior research fellow of the University of Debrecen, Medical and Health Science Center and recipient of the Jedlik Ányos fellowship supported by the TÁMOP 4.2.4. A/2-11-1-2012-0001 grant implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund. Work in the Nagy laboratory is supported by a grant from the Hungarian Scientific Research Fund (OTKA K100196), and TÁMOP-4.2.2.A-11/1/KONV-2012-0023 VÉD-ELEM implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund. This project was also supported by the DEOEC OTKA Bridging Fund.

Regulatory SNPs with possible clinical significance: Genome-wide ChIP-seq based searching and analysis of transcription factor binding sites in human and mouse

Júlia Koller[1], Dávid Jónás[1], Erik Czipa[1], Gergely Nagy[1], Tibor Nagy[2] and Endre Barta[1,2,3]
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Transcription factor ChIP-seq experiments provide genome-wide picture about transcription factor occupancy. Based on this information we can define transcription factor binding sites (TFBSs) de novo. As different cells in different developmental stages and in different environmental conditions utilize different TFBSs in gene regulation we need to process as many ChIP-seq experiments as possible to get a comprehensive picture. SNPs overlapping with TFBSs (regulatory SNPs or rSNPs) can affect gene regulation by altering the affinity of the TF to its binding site. We expect that by knowing more about the TFBSs and rSNPs genome-wide could help in better understanding the transcriptional machinery. Describing rSNPs can also help in the annotation of the SNPs found associated to diseases in GWAS studies.

The aim of our study is to collect and analyze the raw sequencing data from transcription factor ChIP-seq experiments in human and mouse. We have collected the available ChIP-seq raw sequence data from NCBI SRA database and searched for de novo motifs within them using our modified ChIP-seq analysis pipeline. In the TFBSs we found rSNPs that probably alter gene expression. We defined different groups of these rSNPs based on the degree and type of the conservation between other mammalian species at the given position.

We found that these ChIP-seq derived TFBSs are more conserved than the same motifs found in background sequences. Growing the stringency of TFBS processing criteria

yielded the increase of the proportions of the more conserved SNP groups. We found a tendency that if two bases are equally important in the TF binding it is more likely that they both will be conserved evolutionarily.

We compared the rSNPs with SNPs that were connected to diseases by GWAS and are in non-coding regions. We found 129 such SNPs, which are connected with different types of cancer, autoimmune, cardiovascular disease, diabetes, drug metabolism, ageing, quantitative traits, neurological and psychiatric diseases.

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A modular system to study the effect of protease inhibitors on the action of Human Immunodeficiency Virus type 2 (HIV-2).

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Since its discovery in 1986, the human immunodeficiency virus type-2 (HIV-2) along with HIV-1 have infected more than 34 million worldwide. The HIV protease is a homodimeric aspartyl protease that is crucial for the viral life-cycle cleaving proviral polyproteins, hence creating mature protein components that are required for the assembly of an infectious virus. With diagnostic measures and clinically used protease inhibitors (PI`s) focusing on HIV-1, studies of the efficacy of those inhibitors on HIV-2 protease remain widely lacking.

With the help of a wild-type HIV-2 vector backbone and cloning techniques we have developed a cassette system where the efficacy of clinically used protease inhibitors can be studied for various serotypes of HIV-2 protease both in vivo and in vitro. In vitro, the enzyme was expressed and its activity was characterized using high-performance liquid chromatography, the stability of the enzyme was then determined by studying its autodegradation. In vivo, the efficiency of transfection and transduction capability of the cassette system was tested and was not found to differ from the wild-type. Utilizing our cassette system, IC50 and Ki values were measured for some of the most widely used protease inhibitors both in vivo and in vitro. We believe that the combination of in vitro and in vivo studies performed with our modular system will provide an accurate measure of the efficacy of currently used protease inhibitors, bearing in mind the rising incidence of HIV-2 and the scarcity of studies on its protease, the characterization of the inhibition profiling is indeed crucial.

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SECTION 4

C or not C with Hi-C: the 3D organization principles of eukaryote genomes

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The 3D organization of genomes has been investigated using various methods from conventional microscopic to genomic approaches that involve high-throughput proximity ligation assays. The latter "Chromosome Conformation Capture (CCC)"-based techniques (4C, ChIA-PET, Hi-C, GCC) generate huge genomic datasets that unravel the spatial chromatin interaction landscape in an ever increasing number of organisms. The relative simplicity of the methodology (crosslink spatially juxtaposed DNA regions, fragment chromatin to small pieces, ligate neighbouring DNA termini, pair-end sequence ligation junctions) contrasts to the probabilistic nature of the assay and the complexity of data analysis. I will present data showing that we could *(i)* re-build (reproduce) the spatial chromosome architecture of a human lymphoblastoid cell line - calculated from publicly available raw Hi-C datasets, *(ii)* map the distribution of RNA-DNA hybrids - obtained by conventional "one-dimensional" ChIP-Seq - to the 3D genome, *(iii)* identify RNA-DNA hybrids as prominent features responsible for mediating intra- and inter-chromosomal interactions between chromosomal domains.

This work was supported by the European Union (FP7/MCA-CIG) and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.4.A/ 2-11/1-2012-0001 'National Excellence Program', and also by the Hungarian Scientific Research Fund (OTKA-PD).

Mapping the IL4 induced STAT6 transcriptome and cistrome in primary human macrophage like cells

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Macrophages are polarized immune cells that exert sometimes opposing roles depending on their cytokine environment. While pro-inflammatory cytokines give rise to cells that promote the immune response, anti-inflammatory cytokines such as interleukin (IL)-4 lead to the differentiation of cells that attenuate inflammation. Macrophages also regulate lipid metabolism via several members of the nuclear hormone receptor (NR) family, including peroxisome proliferator activated receptors (PPARs), liver X receptors (LXR) and retinoid X receptors (RXR). It is known that cytokines modulate the cells' response to lipid signals but the mechanisms of these events are not well understood.
In the current study we aimed to gain insight into the possible cross-talk between IL-4 and lipid signaling using genome-scale mapping techniques. We determinedIL-4 induced gene expression changes by RNA sequencing and STAT6 binding by chromatin immunoprecipitationsequencing (ChIP-Seq) in human CD14+ monocytes derived macrophage-like cells. We also mapped the active enhancer regions by determining H3K27-

acetylation as well as the binding patterns of PU.1 and RXR, the macrophage specific pioneer factor, and the heterodimerization partner of several lipid regulated nuclear receptors, respectively. Surprisingly, we found that about 2/3rd of the ~1500 IL-4 induced STAT6 peaks co-localized with RXR peaks although bioinformatics analysis was unable to find enrichment of NR motifs within these regions. We also found genes synergistically regulated by the RXR ligand LG268 and IL-4. We are in the process of determining genome-wide gene expression changes by these agents to find commonly regulated genes and determine the functional relevance of their putative cross-talk.
At the end of these studies we hope to better understand how lipid and cytokine signals converge to fine-tune the inflammatory immune response.

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R-loops: Who are they?

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Structures known as R-loops are comprised of RNA-DNA hybrids that are thought to be rare by products of transcriptional processes and/or experimental procedures. However, recent studies have shown that these structures may be abundant in cells and may pose a threat to genome integrity. The quantity and subcellular distribution of endogenous R-loops was investigated in Actinomycin D- and alpha-amanitin-treated Jurkat cells compromised in RNAP/II/III activities. R-loops were detected by indirect immunofluorescence using an RNA-DNA hybrid specific monoclonal antibody (S9.6) and their level was quantified by a laser scanning cytometry (LSC) and confocal laser scanning microscopy (CLSM). In parallel with the detection of R-loops, active RNA synthesis was pulse labeled by an ethenyl-uridine (EU)-based "click" chemistry. Our results show that chromosomal R-loops form in a dynamic, transcription-dependent manner with an average half-life of 26 minutes. Both active transcription and R-loops preferentially localize to the nuclear matrix but they do not co-localize (Pearson correlation coefficient r=0.28), suggesting that R-loops detected by the S9.6 antibody are not related to RNA-DNA hybrids travelling within the substrate binding pocket of RNA polymerases upon transcription elongation. We hypothesize that chromosomal R-loops comprise trans-acting regulatory RNAs present at ~50 kbp intervals that help establish loop-size chromatin domains.

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Quantifying DNA superhelicity and chromatin loop length in eukaryotic cell nuclei

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Studies have shown early on that the DNA of interphase chromosomes in the eukaryotic nucleus is anchored periodically to the nuclear matrix, forming a series of large (50-200 kbp) loops. Since the chromosomal DNA between these fixed attachments points is intact (unnicked), each loop corresponds to a topologically constrained, independent superhelical domain. When isolated nuclei are treated with high salt (2 M NaCl), the histones and other soluble nuclear proteins involved in chromatin-packaging are removed and the DNA bulges out from the nucleus forming a round "halo" surrounding the residual nuclear matrix. The halo, comprising intact, negatively supercoiled (i.e. underwound) DNA loops emanating from the nuclear matrix, can be visualized using fluorescent DNA intercalator dyes. The negatively supercoiled DNA loops can be gradually unwound, relaxed the noverwound, with increasing concentrations of the intercalators, allowing the measurement of the length and superhelical density of the DNA loops.

We have developed a sensitive method for the measurement of DNA superhelicity and loop length of individual cell nuclei, based on the use of a novel, bright fluorescent intercalator and Laser Scanning Cytometry (LSC). The average haloradiat different intercalator concentrations were calculated from the measured haloareas and were plotted against the dye concentration, thus generating a characteristic 'winding curve'. Superhelical densities at different intercalator concentrations were determined by single molecule experiments using magnetic tweezers that could precisely quantify the DNA elongation and unwinding caused by the intercalating dye at the same high salt conditions. Using the intercalator concentration versus superhelical density plot as a standard curve, we were able to convert the dye concentration values of the winding curves to true superhelical densities. By LSC, DNA loop size and superhelicity could be distinguished in the different phases of the cell cycle. Using the fluorescence integrals of the images it was also possible to determine the matrix association of the DNA loops at the different intercalator concentrations.

The DNA loops in the halos appeared completely superhelical throughout the cell cycle, though several thousand nicks were visible in the nuclear matrix associated DNA (Szekvolgyi et al., 2007). This suggests that the halo- and matrix associated DNA represent two distinct chromatin domains that are topologically isolated. Significant changes were observed during the cell cycle in the length and matrix association of the DNA loops of the halo. We have also found differences in the superhelicity and length of the chromatin loops between different cell types and after various treatments, including differentiation induction and replication inhibition. Our current efforts are focused on the determination of the absolute level of superhelicity of the looped DNA after taking nonspecific dye binding also into account.

References: Szekvolgyi L. et al., 2007 PNAS 104, 14964-14969.

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Dendritic cell-associated nuclear protein-1 (DCNP-1), as a potential player in the NFkB pathway

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Dendritic-cell associated nuclear protein-1 (DCNP-1) was first characterized in the peri-nucleus of activated dendritic cells (DC). Recently, DCNP-1 has been detected in the CNS and its expression was found to be associated with major depression disorder (MDD). However, the function of this protein in DC biology and in other peripheral immune cells remained enigmatic and no particular roles or partners could be assigned to it so far.
The goal of our research is to investigate the expression and possible role of DCNP-1 in human monocyte-derived dendritic cells (moDC) at the resting state and in the course of immune responses. In particular we are interested in its participation in the NFkB signaling pathway. To approach these goals we measured the kinetics of DCNP-1 expression at both the mRNA and protein levels in non-stimulated and in activated moDC triggered by various TLR ligands known to stimulate the NFkB pathway. By using the gene silencing technology we also aimed to comparing the activation of DC expressing physiological levels or reduced expression of DCNP-1.
The interplay of the immune system with the central nervous system in general and the specific role of defined cell types and soluble molecules have recently been emerged as a possible mechanism in the development of psycho-neurological disorders. Shedding light on the functions of DCNP-1 is of clinical importance. Implications in understanding the complex pathophysiology of MDD might offer an additional therapeutic approach to this so-far poorly-managed disorder.

IL-4/STAT6 signaling pathway regulates an evolutionarily conserved miRNA signature in macrophages leading to controlled macrophage survival

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MicroRNAs (miRNAs) are short, single stranded RNA molecules, which can inhibit the translation of target genes or can cause mRNA degradation. These molecules play an important role in the regulation of different immune cell differentiation and function, but their expression pattern, regulation and function are not well known during human and mouse IL-4-induced alternative macrophage activation.
We have analyzed global miRNA expression profiles of human un-stimulated and IL-4-stimulated alternatively activated macrophages. Nine miRNAs showed significant minimum

two fold differences between 72 hours un-stimulated and IL-4-stimulated macrophages. We validated and further characterized four miRNAs expression using quantitative PCR. Mir-342 and mir-193b were induced, while mir-125a-5p and mir-99b were repressed during human IL-4-dependent alternative macrophage activation. Three out of four miRNAs including mir-342, mir-125a-5p and mir-99b were also regulated in an IL-4-dependent manner in *ex vivo* differentiated and *in vivo* parasite implantation-induced alternatively activated macrophages in mice. IL-4-mediated regulation of all three miRNAs showed IL-4Rα and STAT6-dependency. Further characterization of intronic mir-342 expression showed that mir-342 and its host gene EVL were regulated in a coordinated manner by IL-4. Furthermore, we found using ChIP quantitative PCR that STAT6 were able to bind directly the regulatory regions of human and mouse EVL genes. Finally, functional characterization of mir-342 suggested that mir-342 over-expression led to decreased macrophage survival via mir-342-dependent induction of apoptosis.
Currently we are in the process of analyzing the potential anti-apoptotic target genes of mir-342 in macrophages using a combination of bioinformatics and biochemical approaches.

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SECTION 5

Lymphocyte subtypes involved in allergic inflammation

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Allergic inflammations involve both humoral (IgE) and T helper 2 (Th2) cell mediated mechanisms. Cytokines released by Th2 cells regulate isotype switching to IgE, mucus hyperproduction, and ***recruitment of eosinophils*** into local ***tissue.*** There is no doubt that Th2 cells are central in the orchestration and amplification of allergic reactions; however, the overall picture of allergic disorders has become more complicated over the past few years. In this lecture, I will focus on recent studies that have provided evidence for a significant contribution of type 2 innate lymphocytes, as well as T cells other than Th2 cells to allergic airway information.

The role of 8-oxoG in allergic airway inflammation

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Introduction: Allergic airway inflammation is characterized by increased expression of pro-inflammatory mediators, inflammatory cell infiltration, mucus hypersecretion, and airway

hyperresponsiveness, in parallel with oxidative DNA damage. The primary target of reactive oxygen species in DNA is guanine due to its lowest redox potential among DNA bases. Although the spectrum of guanine base lesions varies according to the nature of oxidants, 7,8-dihydro-8-oxoguanine (8-oxoG) is the most frequent oxidation product of DNA. In mammals, the intra-helical 8-oxoG is recognized and excised by 8-oxoguanine DNA glycosylase 1 (Ogg1) during base excision repair processes. In a recent study siRNA technology was utilized to deplete Ogg1 expression from the airway epithelium of mice. It has found that decreased Ogg1 expression and thereby 8-oxoG repair in the airway epithelium conveyed a lower inflammatory response after ragweed pollen extract (RWE) challenge of sensitized mice. In this work we further investigated the involvement of 8-oxoG-mediated events in the development of allergic airway inflammation.
Methods: BALB/c mice were sensitized with OVA only and OVA+8-oxoG via intraperitoneal or intranasal routes. Sensitized mice were challenged intranasally with OVA only or OVA+8-oxoG. OVA-specific antibody levels in the serum were measured by ELISA. Total cell counts and differential cell counts in the bronchoalveolar lavage samples were determined on cytocentrifuge preparations after May-Grünwald-Giemsa staining.
Results: We have found that intraperitoneal administration of 8-oxoG parallel with OVA was not able to improve the OVA-specific humoral immune responses. In "conventionally sensitized" (OVA+alum) mice, when 8-oxoG was added to OVA during intranasal challenge, it increased the influx of inflammatory cells into the airways in a dose-dependent manner. The bronchoalveolar lavage samples from mice sensitized intranasally with OVA+8-oxoG contained significantly more inflammatory cells than those from animals sensitized with OVA only.
Conclusions: Our results suggest that a reduction in 8-oxoG levels (inhibition of Ogg1 expression/activity) in the airways may have clinical benefits.

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How does adenosine inhibit LPS-induced proinflammatory cytokine formation in macrophages?

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Recognition of pathogen associated molecular patterns(PAMP) -such as bacterial lipopolysaccharide (LPS) - by specific receptors leads to the activation of macrophages. The classically activated macrophage phenotype is characterized by a genetically determined and pathogen-specific inflammatory cytokine profile. Some of the mediators – such as TGF-β, IL-10 and adenosine - released by macrophages reduce the response therefore they are involved in the termination and resolution of inflammatory process. However, the exact mechanisms involved in the inhibition of overwhelmed inflammation are only partially known. Adenosine has four receptor subtypes: A_1, A_{2A}, A_{2B}, A_3, all of them can be found on macrophages. From the receptor subtypes, the Gs-protein coupled A_{2A} receptor ($A_{2A}R$) plays an important role in the inhibition of inflammation. Previous studies have shown that $A_{2A}R$ deficient macrophages produce higher amount of TNF- alpha (TNF-α) in response to inflammatory stimuli, such as LPS treatment. Our previous results have indicated that not only TNF-α, but also IL-6 and MIP-2 production is affected by a signaling pathway triggered by the activation of $A_{2A}R$.This

pathway influences the LPS-induced activation of MAPK cascade rather than the “classical”, NFkB-mediated TLR4 pathway. Our results show that among the MAPK cascade the JNK1 seems to be affected by $A_{2A}R$ signaling. JNK1is not only involved in the induction of inflammatory mediators, but also plays a role in the regulation of DUSP1 phosphatase which is a key enzyme of the dephosphorylation –and in this way the switching off- of LPS-activated MAPKs (primarily JNK1). The inhibition of $A_{2A}R$-mediated signaling pathway leads to enhanced JNK1 activation and consequentially decreased enzymatic activity of DUSP1. Therefore the inhibition of A2AR pathway results in enhanced pro-inflammatory cytokine production following exposure to LPS.

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Effect of native and oxidatively modified exogenous mitochondrial DNA on the functions of human plasmacytoid dendritic cells

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Introduction: Plasmacytoid dendritic cells (pDCs) are a unique and rare cell population of the immune system. They are specialized for the recognition of nucleic acids of invading microbes by their selectively expressed endosomal nucleic acid-sensing Toll-like receptors (TLRs) such as TLR7 and TLR9. It has been recently demonstrated that extracellular mitochondrial DNA (mtDNA) released from injured or even living cells during inflammation can act as endogenous damage-associated molecular pattern (DAMP) molecule. Mitochondria are evolutionary endosymbionts derived from bacteria and so might carry bacterium-associated molecular motifs so we suppose that extracellular mtDNA is able to induce activation of pDCs.
Methods: mtDNA was extracted from non-treated and oxidative stress-exposed human cells. The levels of the 7,8-dihydro-8-oxoguanine (8-oxoG) in the purified mtDNA, which correlate with the oxidized state of the DNA, were measured by dot blot method. Phenotypic changes of pDCs after mtDNA treatments were monitored by flow cytometry. Cytokine and chemokine secretion of the cells was detected by ELISA.
Results: We found that treatment with mtDNA up-regulated the expression of several cell surface proteins (CD83, CD86, HLA-DQ) on pDCs and increased the type I interferon, TNF-α, and IL-8 secretion. These effects were more apparent when pDCs were treated with high 8-oxoG-containing mtDNA purified from oxidative-stress exposed cells, indicating that 8-oxoG enriched mtDNA sequences arisen under oxidative stress conditions can be more potent activators of the human pDCs than the native ones. In addition, pre-treatment of the cells with TLR9 antagonist (ODN TTAGGG sequence), strongly diminished the ability of mtDNA to induce phenotypic and functional changes in pDCs, indicating that these activation processes were manly mediated through TLR9.
Conclusions: Collectively, our data suggest that the cell-free mtDNA enriched in the extracellular matrix or circulated in the blood-stream after cell injury or inflammation is fully capable of activating human pDCs via TLR9. Furthermore, the oxidatively modified mtDNA generated during the inflammatory reactions may have a greater potential to initiate and maintain of the immune responses.

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mTNF-alpha signaling inhibits LPS-induced proinflammatory cytokine formation by upregulating TGF-β in macrophages

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Objective: Tumor necrosis alpha (TNFα) exists in two isoforms: the soluble sTNFα which is a trimer built up of 17 kDa sized subunits and the membrane-bound 26 kDa sized mTNFα. Increasing evidence suggests that following engagement with TNF receptor mTNFα initiates a reverse signaling pathway, but the details have not been described yet.
Methods: For characterization of TNF production we examined the mRNA levels both in resting and in bacterial lipopolysaccharide (LPS) activated bone marrow derived macrophages by Q-PCR, the amounts of secreted TNFα by ELISA technique, while the amount of mTNFα by flow cytometry. To study mTNFα signaling, mTNFα was crosslinked by coated antibodies and the signaling was studied by the Proteome Profiler Human Phospho-MAPK Array as well as the secreted cytokines were studied by Cytokine arrays.
Results: Our results indicate that following LPS stimulation mTNFα is originated first from the stored cytosolic pool and the *de novo* synthesis contributes to the late expression of mTNFα.TNFα appears first in the membrane, but later it is cleaved by metalloproteases to form sTNFα resulting in a very little steady state mTNFα concentrations on the cell surface. mTNFα reverse signaling induces the production of TGF-beta in mouse bone marrow derived macrophages. In turn, TGF-beta acts back on macrophages and triggers the upregulation of the dual specific phosphatase 1 (DUSP1) and IL-10 via activating the MKK3/MKK6 signaling pathway. As a result triggering mTNFα leads to the downregulation of LPS-induced signaling and the consequent proinflammatory responses (IL-6 production) in macrophages.
Conclusions: Our data indicate that some of the neutralizing anti-TNF antibodies used in human therapy which trigger mTNFα signaling might exert their anti-inflammatory effects via the mTNFα signaling pathway as well.

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SLAMF5 is a regulator of CD40L-induced responses in plasmacytoid dendritic cells

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Background and objectives:
Plasmacytoid dendritic cells (pDCs) regulate both adaptive and innate immune responses. Stimulation of pDCs with Toll-like receptor ligands (TLRL) causes the maturation of pDCs, up-regulates CD40, which increases the ability of pDCs to be further activated via CD40L by T cells. pDCs are capable of promoting the differentiation of Th1, Th2 Th17 or Treg cells,

however the diversity of pDC-responses requires concomitant signaling of various co-receptor molecules including several members of the SLAMF receptor family. Despite the abundance and the clearly strong immune modulatory function of SLAMF receptors, their role in the regulation of pDC functions is poorly understood. We have previously shown that SLAMF1 and SLAMF5 are inhibitory to CD40-induced cytokine responses in myeloid DCs suggesting the existence of a feedback loop controlling inflammatory responses. Here we seek to identify the impact of SLAMF5 on pDC functions induced by simultaneous TLR and CD40 signaling.

Methods and results:

To elucidate the function of SLAMF5 in the Gen2.2 human pDC line we used L929 mouse fibroblast cell lines expressing CD40L alone or together with SLAMF5. These cell lines were cultured with CpG-B or Imiquimod activated Gen2.2 cells hence used as "T cell surrogates", to mimic the effect of co-signaling of these receptors during pDC/T cell interactions. Under these conditions Gen2.2 cells become potent antigen presenting cells expressing high levels of co-receptors (CD80, CD83, OX40L, ICOSL) and produce pro-inflammatory cytokines (IL-6, IL-8, IL-12 and TNFα). The simultaneous TLRL- and CD40L-induced OX40L expression was augmented by the presence of SLAMF5, while production of IL-12 and TNF was decreased. These effects were SLAMF5-dependent as they were reversed by silencing of SLAMF5 expression by specific siRNA. Interestingly, we also found that SLAMF5/SLAMF5 homoassociation increased the capacity of CD40L and TLR7L-activated Gen2.2 cells to support T cell proliferation. The effect of SLAMF5 signaling on instructive signals driving differentiation of various T cell subsets is underway.

Conclusions:

We propose that SLAMF5 is an inhibitory receptor in pDCs controlling exuberant inflammatory responses and thus, may have significant influence on the regulation of tolerogenic versus immunogenic character of pDCs.

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SECTION 6

Dissecting the Genetic and Epigenetic Components of Gene Expression Regulation in the Context of the 1000 Genomes Project.

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„Nature versus nurture" is a century long asked question that could be translated into a similar question: genetic or epigenetic factors are predominantly responsible for individual variations between different persons? Eliminating the variability introduced by sample collection and source of samples is a must in the field and a big challenge for human samples where characterizing the existing variability could be an alternative. The 1000 Genomes Project developed and extensively characterized more than 2500 lymphoblastoid cell lines (LCL-s) and these cell lines can be used to ask the basic question of epigenetic studies: where can we identify the non-DNA encoded individual differences of gene expression regulation?

We decided to study a CEPH pedigree with high genetic variability (CEPH pedigree 1459 for the identification of divergent RXR binding sites in order to map the epigenetic and genetic components of differential transcription factor binding.

All CLC cells from the studied pedigree is expressing both CTCF and RXRalpha in high abundancy (based on interrogation of the Affymetrix datasets published in: Nature 2005 Oct 27;437(7063):1365-9). The CEPH pedigree 1459 has CTCF binding site maps already available (PLoS Genet. 8(3): p. e1002599). In case of CTCF, from 50 000 identified binding sites, 6000 had statistically significant overlaps with SNP-s and roughly 300 of these produced divergent binding.

In our lecture we present data on the human SNP-s found in transcription factor binding sites in the context of the 1000 Genomes data sets.

The project is funded by the Internal Research University grant entitled „Dissecting the genetic and epigenetic components of gene expression regulation in the context of the 1000 genomes project". Balint L. Balint is Szodoray fellow of the University of Debrecen Medical and Health Science Center and recipient of the Magyary Zoltan fellowship supported by the TÁMOP 4.2.4.A/2-11-1-2012-0001 grant, implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund.

Molecular diagnostics of cystic fibrosis: mutational spectrum in Eastern Hungary and the development of a NGS-based method.

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One aim of our investigation was to characterize and update the spectrum of *CFTR* mutations in Eastern Hungary with the help of a representative cohort of 40 CF patients with the classical form of the disease. We utilized the recommended "cascade" *CFTR* mutation screening approach, initially using a commercial assay, followed by examination of the common "Slavic" deletion CFTRdele2,3(21kb). Subsequently, the entire *CFTR* coding region of the CFTR gene was sequenced in patients with yet unidentified mutations. We have identified common CF causing mutations in the Hungarian population with the most common mutations (p.Phe508del, p.Asn1303Lys, CFTRdele2,3(21kb), 2184insA, p.Gly542X, and p.Leu101X), comprising over 93.75% of all CF alleles. Obtained data are applicable to the improvement of DNA diagnostics in Hungary and beyond, and are the necessary prerequisite for the introduction of a nationwide "two tier" CF newborn screening program.

In addition, we analyzed the possibility to implement next-generation sequencing into the routine clinical genetic diagnostic practice. The Roche GS Junior device is working on a pyrosequencing principle and therefore not capable of flawlessly identifying homopolimer sequences longer than four or five bases. By lacking the required analytical sensitivity and specificity it has questionable diagnostic value. However our previous experience suggest, that in the initial phase of sequencing (immediately following the primers) the signal/noise ratio is acceptably high for determining the subtle homopolimer regions, thus constructing primers in the proximity of the repetitive sequences may solve this problem. Experiments are still in progress and we have not reached a conclusion yet, but the intermediate results look promising.

Molecular epidemiological studies in age-related macular degeneration

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Age-related macular degeneration (AMD) is the leading cause of irreversible central vision loss in the elderly in the developed world. Several environmental and genetic factors contribute to the development of the multifactorial disease. We performed a case-control study to assess the risk conferred by known (CFH, CFI, C3, HTRA, LOC387715, ApoE) and candidate genetic polymorphisms (FXIII, Gas6, Mer RTK) on the development of AMD in Hungarian patients. We searched for genetic interactions and for differences in dry and wet AMD etiology. We enrolled 213 patients with exudative, 67 patients with dry AMD and 106 age and ethnically matched controls. Altogether 12 polymorphisms in 9 genes were tested using different molecular genetic methods. We summarized the genotype distributions in the control and patients groups. Unadjusted between-group comparisons and testing of adjusted effects were also performed. Multiple regression models revealed an interesting genetic interaction in the dry AMD subgroup. In the absence of C3 risk allele, mutant genotypes of both CFH and HTRA1 behaved as strongly significant risk factors (OR = 7.96, 95%CI: 2.39 = 26.50, p = 0.0007, and OR = 36.02, 95%CI: 3.30–393.02, p = 0.0033, respectively), but reduced to neutrality otherwise. On the other hand, the risk allele carried a significant risk in the simultaneous absence of homozygous CFH and HTRA1 polymorphisms only, in which case it was associated with a near-five-fold relative increase in the odds of dry type AMD (OR = 4.93, 95%CI: 1.98–12.25, p = 0.0006). Gas6 c.834+7G.A polymorphism was found to be significantly protective irrespective of other genotypes, reducing the odds of wet type AMD by a half (OR = 0.50, 95%CI: 0.26–0.97, p = 0.04). Our results suggest a protective role of Gas6 c.834+7G.A polymorphism in exudative AMD development. In addition, genetic interactions were revealed between CFH, HTRA1 and C3 polymorphisms that might contribute to the pathogenesis of dry AMD.

An alternative way in the diagnosis of aspergillosis; a possible example of translational medicine

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Despite concerted efforts, diagnosis of aspergillosis is still a great challenge to clinical microbiology laboratories. Along with the requirement for high sensitivity and specificity, species-specific identification is important. Although PCR has been used as an aid in the diagnosis of invasive aspergillosis (IA) for almost 3 decades, a lack of standardization has limited the acceptance as a diagnostic tool. The European Aspergillus PCR Initative aims to provide optimal standardized protocols for the widespread clinical evaluation of the Aspergillus PCR to determine its diagnostic accuracy. As members of the European Aspergillus PCR Initiative we circulate and test quality control panels among centers to allow inclusion in disease-defining criteria. In the past years we have developed rapid, sensitive and species-specific qPCR assays for the detection and identification of Aspergillus fumigatus, Aspergillus terreus and Aspergillus lentulus clinical isolates. The assays were designed to target biological marker genes that are exclusively found in a few species of filamentous fungi. The assays have great potential as a molecular diagnosis tool for the early detection of

fungal infection caused by Aspergillus clinical pathogens. The assays were sensitive enough to detect a few genomic equivalents in blood samples.

This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence Program'.

Identification of saliva biomarkers in oral squamous cell carcinoma using targeted proteomics method and multiplexed immunobead-based assay

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Oral squamous cell carcinoma (OSCC) accounts for about 90% of malignant oral lesions which is the 6th most common malignancy with increasing incidence and mortality rate especially in the younger generation. The 5-year survival rate is considerably lower than for cancers of colorectal, cervix and breast origin. Hungarian population occupies the top places of statistics regarding OSCC incidence and mortality figures with 5-fold elevation of overall mortality rate in Hungary since the sixties.

Current diagnostic and screening tools for the OSCC are scalpel biopsy and histopathological evaluation. These techniques require more advanced technical training and skill to prevent false-positive and false-negative results. Delayed detection is likely to be a primary reason for the discovery of the high mortal rate and this supports the need for biomarkers to improve early detection.

The human saliva seems to be an alternative, non-invasive, low-cost source for oral cancer biomarkers and so far different types of genes, proteins, RNAs, miRNAs were examined as potential biomarkers.

The aim of our present research is to develop and validate a targeted proteomic MRM (Multiple Reaction Monitoring) method for examination of previously reported oral cancer protein biomarkers. Using these data we would like to establish a biomarker panel for OSCC screening valid for the Hungarian population.

Analysis of tumor-specific proteomics provides an opportunity for early diagnosis, and detection of OSCC and for a better understanding of behavior of this disease.

Our results show that we could set up and validate a targeted proteomic approach for relative quantification of OSCC related proteins from human saliva. According to our preliminary results CD44 antigen, PS100A9, Profilin-1, Catalase, Thioredoxin, Galectin-3-binding protein, IL-1 alpha, IL-8, TNF-alpha VEGF seem to be useful biomakers for OSCC detection in the Hungarian population.

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SECTION 7

CD55 and CD59 complement regulatory proteins participate in dendritic cell activation by lipopolysaccharide

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CD55 and CD59 are GPI anchored proteins which protect tissues from complement mediated cell lysis. C55 inhibits the assembly of the C3 convertase and promotes the disassembly of preformed C3 and C59 protein complexes to prevent the formation of the membrane attack complex (MAC). It has been shown that in monocytes the CD55 is part of the lipopolysaccharide (LPS) receptor complex and CD59 acts as an LPS receptor in keratinocytes. We hypothesised that the expression of these GPI anchored proteins have an impact on the functional activity of monocyte derived dendritic cells (moDC).
We monitored the expression of CD55 and CD59 proteins in resting, LPS or inflammatory cocktail induced activation of moDCs. The mRNA and surface expression of CD55 was decreased during moDC differetiation whereas the expression of CD59 was increased. As a result of LPS or inflammatory cocktail activation we detected increased of CD55 but the expression of CD59 could be observed only as a result of cytokine cocktail mediated activation. We could not observe significant difference in the response of moDC subpopulations of $CD1a^-$ and $CD1a^+$ cells.
The cell surface expression of CD55 and CD59 could be down modulated by specific siRNA and was associated with the decreased cell surface expression of CD83 in LPS activated cells but had no effect on cytokine cocktail activated moDCs. Inhibition of CD55 and CD59 by specific siRNA resulted in decreased secretion of the pro-inflammatory cytokines TNF-α, IL-6 and IL-12 in LPS activated moDCs. These results suggest that CD55 and CD59 participate in the LPS-mediated activation moDCs and is considered to act as a co-receptors in moDCs.

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Cistromic features of liganded RXR's action in macrophages define a distinct, partly hidden enhancer network

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RXR is an obligatory component of heterodimeric nuclear receptors, but might also act alone to regulate gene expression. RXR signaling is likely to have a major impact in macrophages, but the genomic basis of its ligand-activation is unknown. Genome-wide studies were carried out to map transcriptional changes, nascent RNA production, cistromic interactions and topological domains along with long-range interactions. We identified a restricted network of active enhancers among the 5200 RXR bound genomic regions. Active enhancers are characterized by an increase of enhancer RNA, P300 recruitment and PU.1 release. Using these features 387 liganded-RXR bound enhancers were linked to 226 genes. The long-range enhancers are encompassed in CTCF and Cohesin bordered topological domains and communicate with promoters via stable or RXR-induced loops. A set of angiogenic genes (*Vegfa, Hbegf, Cxcl2, Litaf, Hipk2, Foxo3*) has liganded-RXR controlled enhancers and provides the macrophage with a modular, partly cryptic vasculogenic program.

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Comparative analysis of the P-glycoprotein (ABCB1) expressing and non-expressing JIMT-1 breast cancer cell subpopulations

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Cancer stem cells (CSCs) are tumor cells with stem cell like characteristics including the ability of self-renewal, expression of stem cell markers (e.g. CD44, CD133) and ABC transporters P-glycoprotein (Pgp, ABCB1) and Breast Cancer Resistance Protein (ABCG2, BCRP). There are two methods used for CSC identification: (1) Hoechst 33342 staining (1) and characterization of cancer stem cell markers. Owing to the ABCG2 and/or Pgp expression of CSCs Hoechst can be transported out of these cells resulting in the emergence of a Hoechst negative population ("side population", SP cells). In most cases, CSCs have higher tumor initiating potency and higher expression of tumor markers needed for the invasion and adhesion to form metastases.
Cancer cells often rely on the increased activity of glycolysis rather than on mitochondrial respiration to produce ATP and glycolytic intermediates needed for anabolic cellular processes, and it also provides adaptive advantages for cancer cells to survive hypoxic conditions. To date, little is known about the possible metabolic differences between CSCs and other cells of the tumor.
As a model system we used the JIMT-1 breast cancer cell line, in which we proved the existence of side population cells on the basis of Hoechst staining. The whole JIMT-1 cell population is ABCG2-positive, while only 10-12 % of them express Pgp, but we found no correlation between Hoechst staining and Pgp expression. The Pgp^+ subpopulation showed higher expression of some tumor marker proteins like GLUT-1 glucose transporter and ErbB2, while they had lower CD44 expression. After separation of the Pgp^+ and Pgp^- cells we found that Pgp^+ cells are capable of forming Pgp- cells probably due to asymmetrical division while Pgp- cells were unable to form Pgp^+ cells, however, the proliferation rate of the two populations were similar. Culturing the cells at hypoxic conditions (for 7 days) increased the

number of the Pgp^+ cells by 50%. Interestingly, the Pgp^- cells showed higher glucose uptake compared to the Pgp^+ cells, in spite of their lower GLUT-1 expression and similar hexokinase-I and –II expression.
As a conclusion, we identified a Pgp^+ subpopulation of JIMT-1 cell line with characteristics crucial for chemotherapy resistance and increased metastasis forming potency. Our results also suggest complex differencesin the metabolic processes between the Pgp^+ and Pgp^- JIMT-1 subpopulations.

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Substrate binding and transport by Walker-A mutant Pgps

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P-glycoprotein (Pgp) is an ABC transporter that is able to extrude a large variety of chemotherapeutic drugs from cells, causing multidrug resistance of cancer cells. The protein consists of twelve transmembrane alpha-helices forming the substrate binding site, and two nucleotide binding domains (NBD) involved in ATP binding and hydrolysis. Based on crystal structures of several ABC transporters, Pgp is believed to alternate between an inward and an outward facing conformation, characterized by high and low substrate binding affinities, respectively.

Despite accumulating structural and functional data, it is still unknown how ATP binding and hydrolysis are connected to the conformational changes that allow transmembrane transport. To elucidate partial catalytic reactions, we studied Pgp variants carrying mutations in the conserved Walker A region (K433M and K1076M) of either the N-terminal or C-terminal ABC domains or both. Although mutation of these key residues have been shown to abolish ATPase and transport activity, we found that single mutants possessed a residual drug efflux activity (the double mutant variant was indeed inactive). Confocal microscopic image analysis showed that both the single and double mutant Pgp variants sequester vinblastine-bodipy in the plasma membrane, whereas wild-type Pgp can efficiently catalyze transmembrane transport. Fluorescence cross-correlation analysis proved that the sequestered vinblastine-bodipy strongly co-localize with the mutant Pgp molecules. Since the vinblastine-bodipy staining of the plasma membrane could be competed with Pgp substrates, these results suggest that mutations of the key Walker A lysines stabilize Pgp in the inward open, substrate binding conformation. Using transition state analogs, single mutants could be trapped in the outward open (low substrate affinity) conformation, suggesting that single mutations allow the transition between the two conformations.

Taken together, we show that mutation of a single Walker A lysine is compatible with a residual transport activity. Analysis of the partial catalytic reactions suggests that ATP binding brings about the conformational change needed to switch Pgp from the inward facing to the outward facing conformation.

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The impact of colonic epithelial cell derived stimuli on myeloid cells and T-lymphocytes

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Vitamin A plays an essential role in the maintenance of gut homeostasis, but its interplay with chemokine production in an inflammatory milieu is yet to be fully deciphered. We developed an *in vitro* model system using the human colonic epithelial cell line Caco2, primary blood monocytes derived from human PBMCs and autologous T cells to study the effects of epithelial cell stimulation on dendritic cells and T-lymphocytes. We found that Caco2 cells secrete chemokines CCL19, CCL21 and CCL22 constitutively which are chemo-attractants for professional antigen presenting cells. We also observed that the secretion of CXCL1, CXCL8, Midkine, CXCL16 and CXCL7 is inhibited by ATRA, which also supports the expression of CD14, CCR7 mediated dendritic cell migration to lymph nodes and resulted in enhanced colony stimulating factor (CSF) receptor expression. We also compared the effect of IL-1β and TNF-α on Caco2 cells, we observed that CXCL1, CXCL8 and CCL20 are not secreted by TNF-α. The supernatant of activated Caco2 cells pretreated with ATRA resulted in dendritic cells with increased $CD103^+$ expression indicating a gut-phenotype inducing by ATRA. Polarization of T-cells to Th17 direction was also observed under the influence of colon epithelial cells supernatant.

Inflammatory responses regulated by human monocyte-derived dendritic cells in the gut

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Background: We have established a sensitive *in vitro* culture system for investigating the response of monocyte-derived dendritic cell (moDC) subsets to various commensal bacteria by monitoring the expression of type I/II CD1 proteins, secretion of chemokines, pro-inflammatory and T-cell polarizing cytokines. Under physiological conditions the gut microenvironment is conditioned by all-trans retinoic acid (ATRA) produced by intestinal epithelial cells and $CD103^+$ DCs. To consider the impact of this special microenvironment on moDC-induced T-lymphocyte responses we compared the effects of selected microbes on DC development and T-lymphocyte polarization in the absence and presence of ATRA.
Methods: Monocytes were separated from human buffy coats and differentiated *in vitro* in the presence of GM-CSF and IL-4 with or without 1 nM ATRA for 2 days. Gram(-) (*Escherichia coli from non-altered Schaedler's flora, Escherichia coli 058, Morganella morganii)* and Gram(+) *(Bacillus subtilis)* bacteria were grown in antibiotic-free LB medium and were added to the 2-day moDC cultures for 24 hrs. Activation and phenotypic changes of moDCs was monitored by the expression of membrane CD1a, CD1d, CD83, CD103/integrin αE and CX_3CR1 chemokine receptor by FACS analysis. Culture supernatants of activated moDCs were collected on day 3 of culture and cytokine concentrations were determined by ELISA. The number of IFNγ and IL-17 producing T-cells was monitored by ELISPOT assay.

Expression levels of selected receptors and genes involved in ATRA synthesis was measured by qRT-PCR.
Results: All tested commensal bacteria were able to activate moDCs and induced pro- and anti-inflammatory cytokine secretion. ATRA had a significant impact on the differentiation, inflammatory response and T-cell polarizing activity of moDCs. It decreased the cell surface expression of CD1a while increased that of CD1d and CD103, previously shown to be associated with a shift in moDC functionality. ATRA also enhanced IL-1ß secretion and upregulated the expression of genes involved in ATRA synthesis. Interestingly, these ATRA-induced effects could be counter regulated by the tested microbes. The interaction of microbes with moDC resulted in IL-23 production supporting Th17 polarization of autologous T-lymphocytes and increased the number of IFNγ producing T-cells however, these effects were down modulated by ATRA.
Discussion: In our *in vitro* culture system the $CD1a^{+}CD1d^{-}$ and the $CD1a^{-}CD1d^{+}$ moDC subpopulations respond to and coordinate of the stimuli from commensal bacteria differently to induce T-cell polarization and expansion. Our results also revealed that the tested bacteria modulate the differentiation and activation of moDCs in a dose- and bacterial strain-dependent manner.

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Some new aspects in the signaling of negative selection in thymocytes

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During development in the thymus, immature thymocytes which express self-reactive TCR are eliminated from the developing T-cell repertoire. This negative selection process is mediated by apoptotic signals delivered to thymocytes whose TCR have a high affinity for Self-Antigen/MHC-Antigen Complexes. Among the proteins that are induced by TCR signaling was found the Nur77 transcription factor, which plays a crucial but redundant role in the TCR-mediated thymocyte apoptosis.

Previous work in our laboratory has shown that retionids induce apoptosis in $CD4^{+}$ $CD8^{+}$ thymocytes. I have recently demonstrated that retinoid-induced apoptosis is completely dependent on Nur77. In addition to the already known apoptosis-related Nur77 dependent genes (FasL, TRAIL, NDG-1), we identified two new Nur77-dependent genes, Bid, a BH3-only proapoptotic protein and Gpr65, a pH sensitive receptor, during retinoid-induced apoptosis. In the present work I have investigated whether these Nur77-dependent genes are also induced during negative selection of thymocytes. Here we show the induction of FasL, Bid and Gpr65, but not that of TRAIL or NDG-1 among the known Nur77 dependent genes during negative selection. The induction of these genes was observed in Nur77 null thymocytes as well, in line with the previously reported redundant role of Nur77 with other Nur77 family members. Gpr65 is known to trigger the adenylate cylase pathway and we show that PKA activation leads to the enhanced production of the proapoptotic molecules TNFalphą anḍ Bim. The combined action of FasL and TNFα led to Caspase 8 activation and a Caspase 8-dependent Bid cleavage. In addition, we could demonstrate the Nur77-dependent

induction of STAT1 leading to enhanced Bim expression, and the mitochondrial translocation of Nur77 leading to the exposure of the Bcl-2/BH3 domain.
Our data suggest that both the cell surface death receptor mediated and mitochondrial apoptotic pathway can contribute to the induction of apoptotic cell death during negative selection.

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SECTION 8

Go targetted - the power of targeted proteomics analyses in the study of antimicrobial and immunmodulatory peptides

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Targeted proteomic is a challenging part of proteomics and it is mainly based on the MRM/SRM (Multiple/Selected Reaction Monitoring) scan mode of triple quadrupole-containing mass spectrometers being able to provide with specific identification and quantification of proteins. The MRM scan mode provides high specificity in one hand and quantitative data on the other hand, as the area under the curve of the specific signal is proportional with the amount of the compound entering the mass spectrometer. This scan mode can be used efficiently when the amount of sample available is low – as in case of human tears – because it is more sensitive than the regular MS/MS analysis.
The complex environment on body surfaces coming in contact with the potential pathogens trying to invade our organism consist of the physical and chemical barriers and the commensal bacteria constituting a first line defense system in our body. Major components of the chemical barrier are the constitutively present proteins and peptides from the antimicrobial and immunmodulatory peptide (AMP) family. There are evidences that the AMPs produced in skin may shape the cutaneous microbiota and the host AMP and commensal bacterial AMP may synergize to fight off pathogenic bacterial invasion. Antibacterial immunity in diabetes is impaired, which increases the risk of general and local infections. The antimicrobial peptide composition of tears from patients with normal, non-proliferative and proliferative stages of diabetic retinopathy was examined and the altered antimicrobial peptide cocktail composition was demonstrated.
The presentation gives a short overview on the applicability of targeted proteomics in the analysis of the AMP levels and also shows some other examples when the method was used successfully in the analysis of candidate biomarkers for Alzheimer disease or as a “mass spectrometric western blot”.

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Separation of transglutaminase 2 isopeptidase and transamidase activities

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Transglutaminase 2 (TG2) is a multifunctional and conserved protein in humans taking part in various intra- and extra-cellular processes. TG2 has different enzymatic activities. The best studied one is the Ca^{2+}-dependent transglutaminase activity which covers several kinds of protein modifications: formation of γ-glutamyl-ε-lysine bond between peptides, mono- or polyaminylation by transamidation and deamidation. TG2 has a poorly studied isopeptidase activity when the previously formed isopeptide bonds are cleaved.
Our aim was to separate the transamidase and isopeptidase activities of TG2 applying site directed mutagenesis or special inhibitors.
We have produced TG2 mutants which are isopeptidase or transamidase deficient. Their activities, kinetic parameters, GTP binding have been analysed.
As another approach to separate the different type of transglutaminase activities commercial and newly developed inhibitors were tested on isopeptidase and transamidase activities of TG2. We have found inhibitors which have different effect on the different type of transglutaminase activities.
Altered TG2 catalysed protein cross-linking plays a role in neurodegenerative disorders with the formation of protein inclusions. The pathological protein polymerisations, their inhibition or reversibility will be examined using the isolated functional mutants and the specific inhibitors developing cell models.

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Possible role of transglutaminase 2 activities and variants in aggresome formation

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Protein aggregation is a biological phenomenon in which mis-folded proteins aggregate and clump together. Defects in the clearance machinery leads to accumulation of aggregates in a form termed 'aggresomes' which is implicated in many neurodegenerative disorders. Tissue transglutaminase 2 (TG2) is a ubiquitously expressed, multifunctional enzyme that is well known for its crosslinking and isopeptidase activity. Previous studies have shown that TG2 plays a significant role in modifying the protein aggregates. Using PC12 (Pheochromocytoma) huntingtin rat cell model we have started to analyse how TG2 influence huntingtin aggresome formation using mutants which has only transamidase or isopeptidase activity.

A fluorescence peptide based system to study the isopeptidase activity of TG2 is being developed. This concept if achieved can be applied in larger context to cell models to study the relationship between TG2 and cell aggresome system. TG2-mediated cross-linking of a small fluorescent peptide to a S100A4 protein is visualized. The crosslinked product forming TG2 activity is inactivated with TG2 inhibitor and purified to remove both the inhibitor and fluorescein-PepT26. Finally an isopeptidase assay is done to check the reversal of previously formed crosslinks.
By in silico approach, functional analysis has been done for some rare genetic variants of TG2 and its relation to aggresomes will be studied. Based on the available data the occurrence of SNV's is the lowest in TG2 when compared to the other active transglutaminase family members and human TG2 is under strong evolutionary pressure which prevent the generation of common variants. To study how conserved the evolution and polymorphism of human TG2 we carried out a comparative alignment of humans, mouse and primates sequences of all transglutaminase family members. The differences between the human and mouse sequences are characterized by some triplet changes and mainly in regions of TGM2 outside of so far identified functions suggesting novel gain of function during evolution to humans. Conformational effects due to the triplet changes between mouse and human TG2 is being analysed and later for production of some variants and in vivo / in vitro functional testing will be carried out. It may possibly shed light on the functional differences between transglutaminases of these species and their relevance to human physiology and pathology.

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Quantification of human alpha and beta defensins in colonic epithelial cells by targeted MRM-based proteomics

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Defensins represent an important group of antimicrobial peptides consisting of 16 – 50 amino acids, organized to a structurally conserved compact structure and associated with multiple functions to act in the first line of defense. The three subfamilies of defensins (α, β, θ) differ in their peptide length, location of disulphide bonds between cystein residues, their precursor structures and in the site of expression. They exhibit low immunogenicity, resistance to proteolysis, broad range of anti-microbial activities and based on these activities emerge also as low molecular weight immunomodulators with pharmaceutical potential [1]. We developed an LC-coupled Multiple Reaction Monitoring (MRM) based mass spectrometry method [2] for the analysis of β-defensin 2, β-defensin 3 and α-defensins which may be an alternative way for defensin quantification. The multiplex feature has the advantage over classical ELISAs that the level of multiple proteins can be determined in the same time from one sample having relevance in biology and medicine where the amount of sample to be studied can be a limiting factor. In this study Caco-2 cell lines challenged with IL1β treatment as pro-inflammatory stimuli were used. In cell lysates the level of β-defensin 2 was significantly higher compared to the control samples, both at gene expression and protein level. In cell culture supernatant higher β-defensin 2 and β-defensin 3 amounts were found. Our results show that the developed targeted proteomics method was sensitive and specific for both proteins and could be an alternative quantification method for further immunological studies.

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Retroviral-like Aspartic Protease 1

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ASPRV1 is an aspartic protease which is expressed in skin basal layer located keratinocytes during the terminal period of differentiation. Unlike many retroviral proteases, this enzyme has not been characterized in details. Through the differentiation process keratinocytes become corneocytes, and part of the cornificated barrier which protects the organism from the harmful effects of environment like dehydration, sunburn and infection. Profilaggrin has been reported as a natural substrate of the enzyme and is cleaved in stratum granulosum to filaggrin monomers. These cleaved units bind to keratin-cytoskeleton and the filaggrin-keratin complexes are cross-linked by transglutaminases. Finally, filaggrin go through further degradation to NMF (Natural Moistirizing Factor). This factor also takes care of the skin hydration on the stratum corneum.

Our goal is to characterize ASPRV1 protein from many aspects. We focused on enzymatic, structural and expressional properties of the protein which required *in vitro*, *in silico* and *in vivo* experimental investigations.

GST-tagged proteins were successfully expressed and purified by affinity chromatography. Purified fusion proteins showed enzymatic activity using synthetic oligopeptides using an HPLC detection method. To interpret the specificity data we have prepared a molecular model for the ASPRV1 protease dimer by homology modeling.

The work was supported in part by the TÁMOP 4.2.2.A-11/1/KONV-2012-0023 VÉD-ELEM project (to J.T.) and by the Hungarian Science and Research Fund (K-101591 to J.T.).

Studies on Ty1 and Ty3 retrotransposon proteases

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Ty1 and Ty3 are two retrotransposons from the yeast *S. cerevisiae*. Ty retrotransposons are LTR retrotransposons in which two long terminal repeat (LTR) sequences enclose the*gag* and *gag-pol* open reading frames. LTR retrotransposons and retroviruses show similarity in their life-cycle. The *pol* part of *gag-pol* sequence codes forthree enzymes: protease, integrase and reverse transcriptase.After translation proteases autoprocess themselves,as well as other proteins,from Gag and Gal-Pol.

In our study we have assayed for enzymatic activity recombinant, bacterially expressed Ty1 and Ty3 proteases withsynthetic oligopeptide substrates representing their

natural cleavage sites. We have also measured self-processing and enzymatic activity of a Ty1GAGPR-His_6 fusion protein. Proteases were purified using reversed-phase and Ni-chelate affinity chromatography techniques. A *high*-performance *liquid*chromatography (HPLC)-based assay was utilized to measure activity. While the purified Ty1 and TY3 proteases appeared to be inactive, we were able to detect the self-processing activity of the Ty1 protease of the Ty1GAGPR-His_6 construct.Furthermore, its processed enzyme was also active on oligopeptide substrates. Our results suggest the importance of the Gag part in the folding of the retrotransposon proteases into an active conformation.

The work was supported in part by the TÁMOP 4.2.2.A-11/1/KONV-2012-0023 VÉD-ELEM project (to J.T.) and by the Hungarian Science and Research Fund (K-101591 to J.T.).

SECTION 9 (POSTER SECTION)

Meta-analysis of the ERα CISTROME in various human cell lines

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Chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) allows genome-wide analysis of cis-acting targets of trans-acting factor including estrogen receptor, a nuclear receptor with implications in the prognosis of breast cancer. Nowadays, one of the greatest opportunities in research is the ability to manage large datasets and draw biologically relevant conclusions from them. The main goal of our study is to gain information about characteristics of the estrogen receptor binding events estradiol treatment in four different ERα positive cell lines: MCF-7, T-47D, ECC1 and Ishikawa. We performed meta-analysis on the publicly available ChIP-seq data sets from Gene Expression Omnibus (GEO) and The Sequence Read Archive (SRA) databases. All samples were aligned to the human reference genome (hg19) and an integrated command line analysis for ChIP-seq experiments was performed. We compared ERα peaks and identified the variable and non-variable regions within and among cell lines. Furthermore we determined the possible SNVs (Single Nucleotide Variants) in and near these ERα binding sites. This study provides background data in the correlation between the presence of SNV-s and the differential binding of transcription factors at specific regions of the genome.

The project is funded by the Internal Research University grant entitled „Dissecting the genetic and epigenetic components of gene expression regulation in the context of the 1000 genomes project". Balint L. Balint is Szodoray fellow of the University of Debrecen Medical and Health Science Center and recipient of the Magyary Zoltan fellowship supported by the TÁMOP 4.2.4.A/2-11-1-2012-0001 grant, implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund. [2] Work in the Nagy laboratory is supported by a grant from the Hungarian Scientific Research Fund (OTKA K100196), and TÁMOP-4.2.2.A-11/1/KONV-2012-0023 VÉD-ELEM implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund.

The effect of the HOFI/SH3PXD2B adaptor on the growth of A2058 human melanoma cells

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HOFI/SH3PXD2B (Homologue of FISH) is a membrane associated adaptor containing an N-terminal phox-homology(PX)-domain and four tandem SH3 domains. We and others have shown that HOFI is required for the development of various actin-based structures including lamellipodia, podosomes and invadopodia suggesting its involvement in the regulation of migration, and/or metastasis in transformed cells. The *in vivo* importance of HOFI is supported by two independent animal models. HOFI-deficient mice show abnormal development of the skeleton and white adipose tissue, they also develop galucoma and a profound hearing loss. Here we studied the effect of HOFI/SH3PXD2B in the regulation of cell proliferation using the human melanoma cell line A2058.
HOFI expression in A2058 cells was silenced using two plasmids expressing specific shRNAs (siSTRIKE system Promega) targeting two separate regions of the HOFI gene. A plasmid expressing a shRNA with four altered nucleotides were used as controls. Individual cell clones of HOFI-negative or control cells were serum starved and induced to proliferate under low, normal or high serum conditions (1.0%, 5% or 10% FBS, respectively). Cell numbers at various time points were determined by manual counting. Interestingly, in the presence of HOFI proliferation rates were higher.
Gene silencing experiments require further controls thus, we plan to reconstitute HOFI expression in the above HOFI-deficient cell lines. Reconstitution of HOFI expression using expression vectors encoding the "wild-type" mRNA had failed; therefore we have constructed vectors in which the SiRNA target sequence was altered resulting in an siRNA resistant expression construct. Changes were introduced at the 3rd positions of the codons thus the amino-acid sequence remained unchanged. These constructs have been stably transfected into A2058 cells. Characterization of these cells is underway.
Taken together we show that HOFI is a positive regulator of cell growth in A2058 cells, and we generated several tools that will help uncover the molecular mechanism by which HOFI affects cell growth in tumor cell lines.

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Modulation of early dendritic cell development with lineage determining transcription factors

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Dendritic cells (DCs) can be used as a promising approach to anti-cancer immunotherapy. There are several methods to generate functional DC subsets from various progenitors. These procedures commonly have limitations due to the strict number of available sources. To resolve this obstacle embryonic stem (ES) cells can serve as an outstanding source for clinical applications due to their unlimited expansion capacity and ability to differentiate into numerous cell types. However, for ES cells it remains a major challenge to govern directional

lineage differentiation. To modulate the lineage commitment we intend to modify the transcription programs of ES cell-derived progenitors via overexpression of lineage determining transcription factors.
Here we probed the effects of three DC specific transcription factors (Egr2, Irf2 and Irf8) which have been described as important regulators of DC development. Genetically modified ES cell lines were engineered using our doxycycline (dox) regulated inducible system. We differentiated these ES cell lines to DC progenitors in the presence or absence of dox. Unexpectedly our flow cytomertic analysis revealed that overexpression of Irf2 or Egr2 negatively regulated the formation of myeloid progenitors. However they act at different points of time. Egr2 interfered with the early mesodermal differentiation, in contrast the induction of Irf2 negatively modulate the mesoderm-blood cell transition. These results demonstrate that early induction of DC affiliated factors profoundly modify the mesodermal-myeloid cell development.

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Evolutionary conservation analysis of transcription factor binding sites in human and mouse

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Transcription factors are essential for the regulation of gene expression and, as a consequence, are found in all living organisms. These proteins bind to specific DNA sequences, thereby controlling the flow of genetic information from DNA to messenger RNA, playing a key role in different cell processes like differentiation and metabolism.

The genome-wide investigation of transcription factor binding sites in different organisms provides important information about the evolution of the gene regulation. The development of genomics and bioinformatical approaches allow the global analysis and comparison of these binding sites. The combination of chromatin immunoprecipitation and Next Generation Sequencing technology (ChIP-seq) is a perfect method for TFBS analysis. With bioinformatical analysis of ChIP-seq data, we can extract the localization of the different transcription factor binding sites in the genome.

The National Center for Biotechnology Information's Sequence Read Archive (NCBI SRA) is the central repository of ChIP-seq data. Our raw data was derived from this database and analyzed using bioinformatical methods. We investigated 480 human and 380 mouse ChIP-seq datasets, which was derived from different cell types. We used strict filtering parameters during the analysis to identify the real transcription factor binding sites. We categorized the binding sites based on different criteria such as evolutionary conservation, transcription factor families, distance from the genes and genome wide localization.

The evolutionary conservation analysis was performed using phastCons values. These values are based on a statistical model of sequence evolution called a phylogenetic hidden Markov model, and were downloaded from the UCSC Genome Bioinformatics Site. Using these parameters we proved that the conservation between species correlates on with the base preference of the transcription factor during the binding.

We could compare the transcription factor binding sites between the two species with

the help of the LiftOver program. This program allows converting the binding sites from human to mouse and the other way around. According to our results, these sites show only a low rate overlap between these two species. This means that the evolution of the transcription factor binding sites is more dynamic than it was thought before. The most of the overlapping regions can be found in promoter and intron regions. The low rate overlap between the two species can be explained for example with those transposable elements, which bear transcription factor binding sites.

This research was realized in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 „National Excellence Program – Elaborating and operating an inland student and researcher personal support system", and the TÁMOP-4.2.2.C-11/1/KONV-2012-0010 HPC-NVL program. The project was subsidized by the European Union and co-financed by the European Social Fund.

Chromatin studies for mapping of the Estrogen Receptor αbinding sites in the human MCF7 cell line

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The estrogen receptor alpha (ERα)is a member of the nuclear receptor superfamily, and is known as a hormone-regulated transcription factor.The interactions of ERα and cooperating factors with DNA may influence both gene expression and chromatin organization. The ER-mediated transcription changes play the key role in the development of breast cancer.

The aim of our study is to investigate ERα-DNA interactions in MCF7 cell line. The MCF7 has been used as a model of hormone-responsive breast cancer. For the analysis of DNA coprecipitated with ERαwe used chromatin immunoprecipitation (ChIP) combined with qPCR. We optimalised chromatin fragmentation by sonication and Micrococcal nuclease digestion. We selected tipically ERα-target binding sites, and performed qPCR experiments to detect these genomic regions from ChIP fragments. We compared sonicated and MNase-treated chromatin amounts representing different cell numbers after ChIP with antiH4ac antibody. In order to increase of IP efficiency we titrated the anti-ERα antibody with external control. Finally we compared ERα binding with and without estradiol treatment for these specific regions.

Our study provides insights into optimalisation process of chromatin immunprecipitation for ERα investigation. Further ERα studies may help understanding ER-mediated transcription.

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LXR signaling is inducible in glioma cell lines and is a putative regulator of cell migratory capacity

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Glioblastoma Multiforme (GBM) is the most common and aggressive primary brain tumor. The disease is malignant and invasive; it is one of the most lethal of all cancers. Growth of GBM cells depend on extracellular cholesterol levels. Cholesterol homeostasis is maintained through its uptake, synthesis, and efflux. Expression of enzymes involved in these cellular processes is tightly controlled by Liver X Receptors (LXRs).
Here we examined the activity of LXR pathway and lipid metabolism in human glioma cell line T98 and U251. LXR agonist GW3965 induced the expression of several target genes, including Abca1 (efflux) and Fasn (de-novo synthesis of fatty acids). A long-term treatment with GW3965 resulted in the intracellular accumulation of lipid droplets. Activation of LXR-pathway promoted the expression of GFAP, a marker of mature astrocytes, indicating its potential role in terminal differentiation of astrocytomas. On the other hand, GW3965 increased the level of Mmp2, a well known enzyme involved in degrading extracellular matrix components and enhancing glioma cell migration.
These results indicate a putative role of LXR-signaling in GBM and initiate further in vivo studies.

Zoltan Simandi is junior research fellow of the University of Debrecen, Medical and Health Science Center and recipient of the Jedlik Ányos fellowship. This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence Program'. Work in the Nagy laboratory is supported by a grant from the Hungarian Scientific Research Fund (OTKA K100196), TÁMOP-4.2.2.A-11/1/KONV-2012-0023 VÉD-ELEM and TÁMOP-4.2.2.C-11/1/KONV-2012-0010 HPC-NVL implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund.

Diagnostic accuracy of PCR compared to galactomannan in serum samples of invasive systematic aspergillosis

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Diagnosis of invasive aspergillosis (IA) remains challenging. Signs and symptoms are non-specific and the use of expensive empirical antifungal therapy exposes patients to unnecessary toxicity. We evaluate the performance of our home-brew nucleic acid based diagnostic method targeting species specific biological markers in Aspergillus fumigatus, A. terrus and A. lentulus species compared to the serology testing. The LoD values for our assays range between 5-10 GE in biological samples both in manual and in automated DNA extraction systems. We are performing routine aspergillosis tests in parallel with GM analyses both on whole blood and serum samples in Debrecen. Fungal infections were categorized according to the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group, National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria. Indication for sample taking is the neutropenic fever. DOR was 72. PPV and NPV were 12% and 83% respectively. The Kappa-value was 0,63 which corresponds to a fair agreement.

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Chromosomal R-loops mediate spatial chromosomal interactions

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The spatial organization of chromosomes is non-random and plays important role in gene regulation, DNA replication, genome maintenance and numerous functions in the nuclei. Fluorescent *in situ* Hybridization (FISH) studies revealed that the interphase chromosomes occupy distinct regions in the nucleus. Recent, proximity-based ligation-methods - 3C,4C,Hi-C - enabled the investigation of chromosome structures at a much higher resolution. A revolutional method, Hi-C provides unbiased, genome-wide chromosome interaction profiles for millions of cells at a megabase scale. Here, we used chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) to identify the distribution of R-loops, a type of DNA-RNA hybrids and with the use of publicly available Hi-C data, we have investigated the relationship between chromosomal interactions and R-loops with genome-wide statistical methods (principal component analysis, multivariate correlation and structured interaction matrix analysis) in human peripheral blood lymphocytes. Our results suggest that R-loops are preferentially involved in chromosomal domain-domain interactions.

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Modelling RXR-regulated gene expression dynamics in bone-marrow derived macrophages using steady-state and nascent RNA levels

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Next generation sequencing technologies allow the identification of different aspects of the gene regulation machinery. Nuclear run-on assay combined with massively parallel sequencing methods (GRO-seq) provide complete transcriptional profiles of the RNA polymerases which can be used to estimate transcription speed of nascent RNAs, while whole transcriptome sequencing (RNA-seq) is suitable for the quantification of the steady state level of messenger RNAs.
We developed a differential equation-based model, which attempts to predict the temporal expression profile of steady-state RNA level using nascent and steady-state RNA levels derived from a GRO-seq and RNA-seq time-course data measured on mouse bone marrow derived macrophage cell culture treated with LG268 (synthetic ligand of RXR nuclear receptor) at time points 0, 30, 60, 120 minutes. Interestingly, we found that the profiles of a certain set of genes (with significant change in expression within the measured time interval)

could not be predicted with a constant-decay model. For this subset of genes the model was extended allowing to change the decay constant at a given time.
This phenomenon may be explained by either a rapid post-transcriptional regulation mechanism or by a general post-transcriptional events, which controls the steady state RNA level opposing the effect of external stimuli.

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Structural determination of human Transglutaminase 2 in presence of calcium

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Transglutaminase 2 (TG2) is the member of a large family of enzymes that catalyze protein crosslinking in presence of calcium. Besides its crosslinking activity, TG2 also has several other biochemical activities such as isopeptidase, deamidase and amine incorporation into the proteins and calcium is essential for catalyzing all of these activities. It is clear from the biochemical experiments that TG2 binds six calcium ions which lead to large conformational change in the protein structure. Crystal structure of TG2 has been solved in presence of GTP/GDP and an active site inhibitor. Calcium and GDP/GTP inversely regulates transamidation activity of TG2. When TG2 is bound to GTP it is in a closed conformation, which corresponds to inactive form of the enzyme while when it is bound to calcium, it gets activated and adopts an open conformation. Crystal structure of TG2 bound to inhibitor also adopts an open conformation and it is speculated that it is the active conformation of TG2. Until now, no TG2 structure is available which is bound to calcium hence it is difficult to predict whether inhibitor bound structure is the real active form of the enzyme. Therefore, the main aim of our project is to crystallize TG2 in presence of calcium.
TG2 besides crosslinking other proteins, also gets crosslinked it self in presence of calcium hence it has always been difficult to purify TG2 and crystallize it. We use wild type TG2 as well as active site mutant (C277S) for crystallization trials. The active site mutant lacks transamidation activity. Both the constructs were purified using cobalt affinity column and then gel filtrated to remove the aggregated and crosslinked proteins. Before loading the protein on the gel filtration column, it was incubated with 0.5 mM $CaCl_2$ for an hour. Gel filtration buffer also contained 0.5 mM $CaCl_2$. TG2 was concentrated to 20 mg/ml and crystallization screens were performed in 96-well-plates using PACT premier, JCSG+ and Structure1&2 screens available from Molecular Dimensions. There was good precipitation and phase separation but unfortunately we couldn't get any crystals. We designed another construct of TG2 with a short N terminal linker and a TEV cleavage site. The protein was purified as before and the N'terminal tag was removed by treating it with TEV protease overnight. The protein was subsequently purified again and gel filtered in presence of 0.5 mM $CalCl_2$ and and concentrated to 18 mg/ml. PACT screen gave some positive hits with beautiful rod shaped crystals. The conditions have to be optimized further to get stable crystals, which could be used for X-ray diffraction in future.

Activation of retinoid X receptor induces a proangiogenic macrophage phenotype in vitro

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Tumors develop in a complex tissue microenvironment containing a wide spectrum of host cells such as fibroblasts, endothelial cells and various immune cells. Among immune cells, tumor-infiltrating macrophages significantly contribute to tumor progression by inhibiting anti-tumor immunity, increasing the invasive potential of tumor cells, and enhancing tumor angiogenesis. It is therefore of importance to identify signaling pathways, which are involved in the initiation of these effects. Retinoid X receptor (RXR), a member of the family of nuclear receptors, is activated by vitamin A metabolite 9-cis-retinoic acid and fatty acids and acts as the obligate heterodimeric partner of several nuclear receptors, like PPARγ, LXR, or RAR, thus mediating diverse signaling pathways simultaneously.
Our aim was to examine the effect of RXR activation on macrophage gene expression *in vitro*, and identify RXR-responsive gene networks with a potential role in generating an anti-/pro-tumoral macrophage phenotype. We also aimed to study tumor development in mice with RXRα/β-deficient macrophages.
Mouse bone-marrow-derived macrophages (BMDM) were treated with pan-RXR agonist LG268. Changes in mRNA expression induced by specific RXR activation were detected by microarray analysis. The angiogenic potential of RXR-activated BMDMs was assessed by human umbilical vein endothelial cell (HUVEC)-based tube formation assay. Tumor development in Lys-M-Cre $RXR\alpha^{flox/flox}\beta^{-/-}$ animals was studied in B16-F10 mouse melanoma xenograft model.
Specific RXR activation in mouse BMDMs induced a pro-angiogenic gene network including VEGFα, one of the crucial mediators of tumor angiogenesis. Using a tube formation assay we found that RXR-activated BMDMs induce angiogenesis *in vitro*. However, primary tumor development in Lys-M-Cre $RXR\alpha^{flox/flox}\beta^{-/-}$ mice was unimpaired in our xenograft tumor model.
Our results suggest that *in vitro* RXR activation generates a macrophage phenotype possessing pro-angiogenic capacity. Based on our *in vivo* observations we hypothesize that this signaling pathway is presumably either inactive or silenced in the macrophages residing in the tumor microenvironment.

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Enhanced adipoyte differentiation of mouse embryonic stem cells

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Recently, ascorbic acid (AsA) has been identified as an epigenetic regulator that enhances various cellular differentiation processes; however the mechanism of action remained largely unknown. AsA is known to act as an antioxidant; and it also plays an essential role as a cofactor for many enzymes, including prolyl-4-hydroxylases (PHDs). Prolyl-4-hydroxylation is necessary for oxygen-dependent protein stability of hypoxia-inducible transcription factors (HIFs). Pharmacological inhibition of PHD activity during the initial stages of adipogenesis abrogates the formation of adipocytes.
We here demonstrate that addition of AsA to the adipogenic cocktail promote the differentiation of mouse embryonic stem cells (ESCs) and primary mouse embryonic fibroblasts (PMEFs) to adipocytes. Even in low concentration of AsA (40 microM) significantly improved adipocyte differentiation of PMEFs and resulted in an increased expression of fatty acid binding protein 4 (Fabp4). Addition of dimethyloxaloylglycine (DMOG), a PHD inhibitor, activated the hypoxic response pathway in PMEFs and could also induce the expression level of Fabp4. Moreover, we found evidence that addition of AsA also activates hypoxia inducible mechanisms of PMEFs.
These results suggest the existence of a link between AsA and low oxygen condition and implicate their regulatory function during formation of adipocytes.

Zoltan Simandi is junior research fellow of the University of Debrecen, Medical and Health Science Center and recipient of the Jedlik Ányos fellowship. This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence Program'. Work in the Nagy laboratory is supported by a grant from the Hungarian Scientific Research Fund (OTKA K100196), TÁMOP-4.2.2.A-11/1/KONV-2012-0023 VÉD-ELEM and TÁMOP-4.2.2.C-11/1/KONV-2012-0010 HPC-NVL implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund.

Studies on the human NOD-like receptor family CARD domain containing 5 (NLRC5) protein

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NOD-like receptor (NLR) family member proteins have important role in the regulation of innate immune responses, as these pattern recognition receptors are involved in the recognition of microbial- and danger-associated molecular patterns in vertebrates.

The NOD-like receptor family CARD domain containing 5 (NLRC5)protein is a unique member of NLR protein family due to its high molecular mass (1866 amino acid length, 204kDa), the presence of an untypical caspase activation and recruitment domain (uCARD) effector domain and an unusually high number of leucine-rich repeats (LRRs).NLRC5is involved in the regulation of MHC I transcription, therefore, it is also

referred as MHC class I transactivator (CITA), furthermore, it is involved in the regulation of various signal transduction pathways.NLRC5 is an intensively studied receptor but its direct ligand is unidentified and its involvement in the inflammasome activation is still controversial.

We have recently published homology models for both the monomeric and a homo-heptameric full-length human NLRC5protein. The aim of this study is to correlate biochemical findings with the proposed models.

We have expressed Flag-tagged NLRC5 protein in 293T cells. Anti-Flag and anti-NLRC5 antibodies were tested for the identification of the fusion protein by immune detection. The overexpressed protein was identified successfully by anti-Flag antibodies, while the tested anti-NLRC5 antibodies appeared to be less effective in the identification of the fusion protein. As NLRC5 shuttles between the nucleus and the cytosol, Flag-tagged NLRC5 was detected in both cellular fractions by Western blot. Gel filtration of total cell lysates was performed to determine the molecular weight of NLRC5 and results suggest potential oligomerization of the overexpressed NLRC5.An MRM-based method was also designed which was found to be specific for the NLRC5, and using this method we proved that the transfected cells could express the NLRC5 protein contrary to the non-transfected cells. This method can support our functional studies in the future to study the expression of NLRC5 protein in various circumstances and explore how NLRC5 is involved in immunological function.

The work was supported in part by the TÁMOP 4.2.2.A-11/1/KONV-2012-0023 VÉD-ELEM project (to S.B and J.T.) and by the Hungarian Science and Research Fund (K-101591 to J.T.). S.B. is receiver of LajosSzodoray Postdoctoral Fellowship and Janos Bolyai Postdoctoral Fellowship. A.V. is receiver of Ányos Jedlik Predoctoral Fellowship (TÁMOP4.2.4.A/2-11-1-2012-0001; A2-JÁDJ-12-0186).

Analysis of gene copy number variations in malignant hematological diseases

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Copy Number Variation:
Copy number variation of certain genes plays an important role in malignant hematological diseases. In cancer pathogenesis, tumor suppressor genes or oncogenes may have a reduced or increased expression due to copy number variation. We can assign certain genes to a particular phenotype using gene mapping and other techniques. It has been revealed recently that 12% of the human genome is affected by copy number variation, meaning full or partial gene duplications or deletions.
We examined the following malignant hematological diseases:

- acute lymphoblastic leukemia
- acute myeloid leukemia
- chronic lymphocytic leukemia
- chronic myeloid leukemia
- multiple myeloma
- myelodysplastic syndrome
- non-Hodgkin lymphoma

WT1 gene:
Full name: Wilms Tumor 1
Location: Chr.11 p13

Function: transcription factor, urogenital system development, cell survival and cell mortality
Function defect: Wilm's tumor, Denys Drash syndrome
EphA3:
Full name: Ephrin receptor A3
Location: Chr.3 p11
Function: tyrosine kinase receptor, bind ephrin A protein, cell immobilization, nervous system development, important role in hematopoiesis, chemotaxis of T lymphocyte, vascularization pattern forming.
CHST 9:
Full name: Carbohydrate (N-Acetyl-galactosamine 4-0) Sulfotransferase
Location: Chr.18 q11
Function: the protein is a sulfotransferase in Golgi membrane, posttranslational modification, cell-cell interaction signal transduction, embryonic development.
BS69:
Full name: Zinc Finger, MYND-Type Containing 11
Location: Chr.10 p15
Function: binding A1 protein of adenovirus in nucleolus, transcriptional repressor, chromatin remodeling
Function defect: ovarian tumor, fibrodysplasia ossificans progressiva
ZMAT4:
Full name: Zinc Finger, Matrin-Type 4
Location: Chr.8 p11
Function: transcriptional factor with DNA and zinc binding region.
The experimental process:
The human whole blood samples arrived from DEOEC Department of Internal Medicine. In the first step we extracted DNA from the samples. Then we tested the aforementioned genes and a reference housekeeping gene (Rnase P) using each DNA sample by the qPCR. In the next step we transferred the PCR data to a computer program (CopyCaller). It is used to calculate the copy number of the genes: the program compares quantitative data of each target gene to the reference gene. In case a copy number alteration is detected, a gene expression experiment is also performed.
Purpose:
We want to confirm the relationship between the copy number variation of genes and the hematological diseases. In the future, medical science may use these genes as therapeutic or diagnostic targets.

Inhibitory role of NLRX1 in human dendritic cells immunosupressed by MSCI

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Mesenchymal stromal cells (MSC) have the potential to modulate the functional activities of inflammatory cells among them professional antigen presenting cells (APC) such as dendritic cells (DC). In this study we tested the effects of a previously characterized MSC-like cell (MSCl) on monocyte-derived DC (moDC). To approach this goal we co-cultured moDC and

MSCl cells for four days at a ratio 5:1, respectively and then activated the cells with PolyI:C acting as a common ligand of the intracellular pattern recognition receptors (PRR) RIG-I and MDA5. To analyse gene expression patterns and cytokine secretion the co-cultured cells were separated after the activation process.
We observed significant changes in the level of activation of moDC by measuring the cell surface expression of CD1a and CD83. The concentration of inflammatory and immunosuppressive cytokines was measured in the supernatant of cell cultures. Furthermore, we also monitored the gene-expression profiles of the RIG-I, MDA5, IRF3, IFNβ and NLRX1 genes. The expression of CD1a cell surface molecule on moDC varied among different blood donors and could be correlated to the inflammatory or tolerogenic activity of moDC. CD1a expression levels did not change as a result of PolyI:C-induced activation, but its appearance was completely inhibited in the presence of MSCI cells. The expression of CD83 also decreased indicating the inhibitory role of MSCI cells. The concentration of the inflammatory cytokine IL-6 and the chemokine IL-8 measured in the supernatant of the co-cultured cells was higher than that measured in the supernatant of the moDC cultures. Presence of the MSCI cells also inhibited the secretion of TNFα, IFNγ and IL-12 by moDC thus preventing the activation of IFNγ producing Th1 cells. Presence of MSCI cells also resulted in increased T_{reg} cell expansion and IL-10 production in separated moDC.
When the possible inhibitory effect of the NLRX1 protein was studied we found that it contributed to the down modulation of the inflammatory response. According to our results the presence of this Nod-like receptor family member is necessary for the production of IL-10 secretion by moDC. Upon activation by PolyI:C the mRNA expression of NLRX1 was increased, whereas the mRNA expression of RIG-I and MDA5 was decreased in separated moDC. Our results show that MSCI cells can exert immunomodulatory effects and are able to inhibit inflammatory reactions. We also demonstrated that the NLRX1 protein contributes to this regulation.

Setting up epigenetic studies on twelve B-lymphoblastoid cell lines of the 1000 Genomes Project

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Chromatin immunoprecipitation (ChIP) is widely used for almost 25 years to detect specific protein-DNA interactions and histone modifications in vivo (Solomon et al, Cell 53, 937-947, 1988). Using deep sequencing methods (ChIP-seq)to map global binding sites, the cistrome of a given transcription factor can be determined. Non-coding, regulatory SNPs (rSNPs) affecting transcription factor binding motif scan change gene expression profiles and therefore are expected to have an important role in the molecular characterization of disease-associated complex traits

Our aim is to analyse heritable genome-wide binding patterns of CTCF and RXR on 12 individuals of a family spanning three generations(CEPH pedigree 1459)by ChIP-seqand to investigate epigenetic marks that are correlating with context-dependency. Complex genomic datasets of the samples are available in the 1000 Genomes Project's database.

As preliminary experiments, we prepared a tiered biobank system of the 12 cell lines creating seed stocks of consistent quality. Positive control regions for ChIP-qPCR validations were selected by reanalyzing publicly available ENCODE ChIP-seq data from LCLs. Both

sonication and micrococcal nuclease treatment were optimized to gainfragment sizes that are suitable for ChIP-qPCR and ChIP-seq. Human RXR and CTCF-specific antibodies were titrated to determine the saturating amount of antibodies for subsequent large-scale experiments.

The project is funded by the Internal Research University grant entitled „Dissecting the genetic and epigenetic components of gene expression regulation in the context of the 1000 genomes project". Balint L. Balint is Szodoray fellow of the University of Debrecen Medical and Health Science Center and recipient of the Magyary Zoltan fellowship supported by the TÁMOP 4.2.4.A/2-11-1-2012-0001 grant, implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund.[2] Work in the Nagy laboratory is supported by a grant from the Hungarian Scientific Research Fund (OTKA K100196), and TÁMOP-4.2.2.A-11/1/KONV-2012-0023 VÉD-ELEM implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund.

Compounds enhancing intracellular cAMPlevels increase the expression of transglutaminase 2 in mouse thymocytes

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Previous work in our laboratory has shown that transglutaminase 2 (TG2), a multifunctional protein is induced during in vivo, but not in vitro apoptosis of thymocytes indicating that factors present in the tissue environment are required for the process. Recently our laboratory has found that retinoids and TGFalpha released by engulfing macrophages are involved in the process. Since adenosine and PGE_2, which are also produced by macrophages during engulfment of apoptotic cells, trigger the adenylate cyclase pathway, we decided to investigate the possible involvement of the adenylate cyclase pathway in the TG2 induction. Our data indicate that compounds enhancing intracellular cAMP levels, alone or with combination with retinoids and TGFalpha can contribute to TG2 expression in dying thymocytes.

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Activation of LXR signaling modulates neural differentiation of mouse embryonic stem cells

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Liver X Receptor α and β (LXR α, β) are members of the nuclear receptor family expressed in the central nervous system. Deletion of LXRs reduces cell cycle progression and ventral midbrain neurogenesis, resulting in decreased dopaminergic neurons at birth. The mechanism, how LXRs control neural cell fate specifications remained largely unknown.
Embryonic stem cells (ESCs) can be induced to differentiate into various populations of neural progenitors and terminally differentiated neural subtypes.

In this study, we determined the genome-wide occupancy of RXRs, obligate heterodimer partners of LXRs, in ESCs and terminally differentiated neurons by ChIP-seq. Our motif analysis indicates the enrichment of DR4 motif, the canonical LXR/RXR heterodimer binding site in differentiated neurons. RXR binding on the close proximity of several genes, known to be under direct regulation of LXR/RXR in other cell types, were identified in neurons. These genes, including Abca1, Abcg1, Fasn and Scd1, showed increased expression level upon LXR-specific ligand treatment in terminally differentiated neurons. Neural differentiation of ESCs in the presence of LXR agonist GW3965 also increased the level of these genes; moreover it resulted a disregulated expression of several neural markers, such as Tuj1, Dcx, Lmx1a, Mapt and Th.
These results confirm the importance of LXR/RXR-pathway in the regulation of neural differentiation and give a more mechanistic insight how LXR is involved in neural cell fate specification.

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The effects of the wild type *Lactobacillus casei* BL23 and its peptidoglycan-hydrolase mutants on human monocyte-derived dendritic cell functions

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Lactobacilli are facultative anaerobic, rod-shaped Gram-positive bacteria with pro-biotic potential. The dominant cell wall component of $Gram^+$ bacteria, among them *Lactobacilli* is the outer peptidoglycan (PG) layer, which plays role in the maintenance of bacterial shape and integrity. PG is the target of specific peptidoglycan-hydrolases, which degrade the PG chain synthesized by the bacteria themselves at specific cleavage sites. As a result of PG degradation the glycan-peptide subunits can be recognized by extra- and intracellular receptors of human monocyte-derived dendritic cells (moDCs).
In our experiments we compared the functional activities of live wild type *L. casei* BL23 bacteria and their peptidoglycan-hydrolase mutants. We showed that the genetic modification of the cell wall modulated the phagocytic activity and the inflammatory response of moDCs. We also found that the bacteria induced moDC activation shown by the increased expression of multiple activation markers of moDCs. In line with moDCs activation the secretion of pro- and anti-inflammatory cytokines and the IL-8 chemokine was shown to be increased. Based on ELISA measurements the wild type *L. casei* BL23 bacteria were more active in inducing the secretion of inflammatory cytokines and IL-8 as compared to peptidoglycan-hydrolase mutant bacteria. Furthermore, the moDC-mediated polarization of T-lymphocytes induced by *Lactobacilli* could be detected by the ELISPOT assay. Our results indicate that *L. casei* BL23 wild type and mutant bacteria differ in their ability to activate moDCs and polarize T cells to Th1 or Th17 directions.

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Genome-wide analysis of Hepatocyte nuclear factor-1 & -4 binding sites in correlation with maturity-onset diabetes of the young

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The genome-wide association studies have been stared as one of the most advanced biomedical discovery methods, and ever-since widely applied for the better understanding of genetic components of various diseases including diabetes. A major limitation of these studies consists in the difficulty to explain the correlation between non coding SNP-s (Single Nucleotide Polymorphisms) and the diseases investigated. Hepatocyte nuclear factor-1 alpha and beta, and -4 (HNF1A, HNF1B, HNF4) mutations have been discovered to be the cause of monogenic diabetes, especially in maturity-onset diabetes of the young (MODY). However little is known about the presence of this disease in association with different motifs of HNF-binding sites. Our aim is to investigate this question on a genomic scale and identify the degree of correlation between HNF binding site variants with MODY. The raw ChIP-seq data of published studies was selected from 5 different sequence repository sites and filtered through a set of curration criteria before being processed by our analysis pipeline (Endre Barta 13-17. EMBnet. Journal, 2011). Here we present the binding sites in the investigated tissues and the identified SNP-s in the HNF binding sites. Further laboratory experiments are needed for the validation of the differential binding sites and the impact of the SNP-s on the binding of HNF proteins to their targets.

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Purification and *in vitro* characterization of a mutant capsid protein of HIV-1

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The HIV-1 capsid protein was found to be an *in vitro* substrate of the protease, and its proteolytic cleavage may contribute to the disassembly of the viral core in the early stage of infection. Since the virus entering the cells contains the active protease, its activity could be boosted by the acidic pH of the endosomes and the protease-mediated cleavage of viral proteins (including capsid proteins) may be important for the preparation of functional preintegration complexes. Our previous studies showed that some of the introduced mutations of the capsid cleavage sites substantially changed the secondary structures of the protein. Moreover, these mutants also lost their ability to bind the human peptidyl prolyl isomerase, cyclophilin A (CypA) protein. Since the presence of the CypA is essential in the mature virion

for viral infectivity, the loss of the binding ability may contribute to the previously observed negative effects on the infectivity of the mutant virions. In this project we desired to study the effects of mutation in a previously described secondary cleavage sites of the capsid protein.

In this work we have introduced the L205A amino acid substitution into a histidine-tagged recombinant capsid protein in order to examine the effects of this mutation on the proteolytic cleavage efficiency and CypA binding *in vitro*. We have determined that this amino acid change impaired the proteolytic cleavage efficiency of the capsid protein by the protease. Moreover, the introduction of this cleavage site mutation also impaired the CypA binding ability of the protein, suggesting that it may also inhibit the infectivity of the virions harboring this mutation.

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SECTION 10

Origin and function of macrophages in tissue regeneration

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Restoration of tissue structure and function after injury is vitally important. Macrophages have been shown to regulate tissue regeneration by unknown mechanisms. We mapped the origin and function of macrophages in a mouse model of skeletal muscle injury by a combination of genetic crossings and transcriptomic analysis of the involved macrophages. We identified the origin and gene expression pattern of elicited tissue macrophages during regeneration and mapped the molecular pathways governing macrophage function in regeneration. Our results provide the first description of gene expression patterns in regenerative macrophages and establish molecular links between cellular metabolism and tissue regeneration.

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Macrophages engulfing apoptotic cells produce retinoids to enhance phagocytosis of apoptotic cells

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Retinoids acting via retinoid (RAR) and retinoid X (RXR) receptors have been reported to modulate several functions of mononuclear phagocytes, such as proliferation and phagocytosis of Fc-opsonized particles or yeast cells, but their effect on the apoptotic cell uptake has so far not been investigated. Here we report that following treatment of bone marrow derived macrophages with various natural and synthetic RAR or RXR ligands we detected enhanced apopto-phagocytotic capacity and increased expression of the phagocytosis receptor Mertk, Stabilin 2, TIM4 and CD14, opsonin C1qb, lipid transporter ABCA1 and the integrin beta3 co-receptor transglutaminase 2 genes. Previously it was reported that apoptotic cells promote their own clearance by triggering the lipid sensing LXR receptor in phagocytes following their uptake. This observation was explained by demonstrating that LXR stimulation upregulates the expression of the phagocytosis receptor Mertk, the integrin co-receptor transglutaminase 2 and RAR□. Since RAR□ is an LXR target gene, and previously we detected increased expression of retinoic acid (RA) producing RALDH1, 2 enzyme expression in LXR agonist treated macrophages, we hypothesized that LXR receptor activation might initiate RA production and signaling which could contribute to the observed phagocytosis enhancement following LXR activation. In line with this, LXR agonist treatment upregulated phagocytosis related genes, which were partially overlapping with the retinoid induced ones. Blocking RA production during LXR activation attenuated the phagocytosis enhancement and abolished the induction of retinoid dependent genes suggesting endogenous retinoid production. Indeed, with HPLC technique and using RARE lacZ transgenic mice, in which the lacZ expression is dependent on endogenous retinoid production, we detected the presence of RA in thymus and peritoneal macrophages of LXR agonist injected mice, respectively. Increasing evidence suggests that impaired phagocytosis of apoptotic cells contributes to the pathogenesis of various autoimmune diseases. Our results identify molecular targets to enhance phagocytic capacity of macrophages.

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Characterization of inflammatory reactions of human macrophages to differentiating adipocytes

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Nowadays obesity is an epidemic health problem worldwide, enhancing the risk for metabolic disorders such as type 2 diabetes, nonalcoholic fatty liver disease, metabolic X-syndrome and cardiovascular diseases. Hypertrophic adipose tissue is associated with a rise of free fatty acids (FFA), adipokines and proinflammatory molecules (IL6, TNFalpha, MCP-1). Weight

gain correlates with adipocyte size expansion and an increased number of dying adipocytes. The elevated levels of FFA and proinflammatory cytokines attract monocytes into the hypertrophic adipose tissue. These recruited monocytes differentiate to activated macrophages, which are situated around dead adipocyts, in a “crown like” structure, release more pro-inflammatory cytokines, which cause an inflammatory vicious cycle in white adipose tissue.
The types of adipocyte cell death and their connection with macrophages is not completely characterized yet. We have created a human *in vitro* model for adipocyte cell death and a phagocytosis assay involving human adipocytes and macrophages. As an adipocyte source, we use SGBS human preadipocyte cell line and precursor cells isolated from human subcutaneous adipose tissue differentiated *in vitro* into adipocytes. The macrophages are derived from primary human monocytes. We have characterized the morphological changes and cell death types of adipocytes during differentiation. The lipid content of differentiating adipocytes and their cell death profile has been measured on a time curve by laser scanning cytometry. We studied the cytokine profile during phagocytosis of adipocyte corpses by macrophages. The ideal time point for studying interaction of adipocytes with macrophages and the resulting pro-inflammatory effect has been determined.
We detected lipid accumulation, shrinkage of nuclei and an increasing level of spontaneous adipocyte cell death during the progress of adipogenic differentiation. Dying adipocytes show apoptotic feature due to Annexin V positivity and their anti-inflammatory effect. Significant phagocytosis of adipocytes, dying spontaneously could be detected. Coincubation of differentiated adipocytes and macrophages leads to a unique type of IL6 production and secretion which is a phagocytosis dependent. These data may lead to better understanding of the complex regulatory processes which take place between differentiating/dying adipocytes and macrophages.

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Involvement of LXR receptor in theglucocorticoid-induced enhancement ofapoptotic cell phagocytosis

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The rapid and effective clearance of apoptotic cells by macrophages is critical to prevent the development of inflammation and autoimmune diseases. Previously we have shown that the dexamethasone-induced in vivo apoptosis of thymocytes is accompanied by enhanced retinaldehyde dehydrogenase (RALDH1 and2, two retinoic acid synthesis specific enzymes) expression in engulfing macrophages, indicating the production of retinoids during phagocytosis. In addition, dexamethasone alone can also enhance the expression of RALDH1 in macrophages. Since it is known that dexamethasone enhances the phagocytosis of apoptotic cells by macrophages we decided to test whether retinoids participate in the process. We show that dexamethasone induces the immediate expression of Mer tyrosine kinase, a phagocytic receptor, LXR/RXR nuclear receptor and CYP27, an enzyme that produces an endogenous ligand for the LXR receptor. LXR participates in the long term maintenance of Merexpression, as well as during long term leads to enhanced RALDH expression, retinoid production and enhanced expression of retinoid dependent phagocytic genes. As a result,

inhibition of retinoid production attenuates dexamethasone induced long term phagocytosis of apoptotic cells.

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Clearance of dying RPE cells by professional and non-professional phagocytes as in vitro model for age-related macular degeneration (AMD)

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Purpose: Retinal cells die throughout our lifetime by different cell death modalities including apoptosis, anoikis, autophagy and necrosis. Inefficient removal of the dying cells by professional and non-professional phagocytes can lead tocellular debris formation and development of AMD - the leading cause of blindness in the elderly worldwide.We aimed to study the clearance ofautophagic dying RPE cells by these phagocytes as a model for dry and wet type of AMD, respectively.

Methods: Autophagic cell death was induced by serum deprivation and H_2O_2co-treatment in ARPE-19 cells. Annexin-V FITC/PI flow cytometric assay was used to determine the cell death rate, while autophagy was detected by Western blot quantification of LC3 II/LC3 I ratio and p62 expression, transmission electron microscopy (TEM) and fluorescence microscopy of GFP-LC3 transfected RPE cells. The clearance of autophagic dying cells by non-professional (living ARPE-19/hRPE cells) and professional (human blood monocyte-derived macrophages) phagocyteswere quantified using flow cytometry.

Results: An increasing percentage of phosphatidylserine positive or dying RPE cells were observed in a time- and concentration dependent manner upon H_2O_2treatment. Paralelly, an induction of autophagy could be detected within 2hrs of treatment with 1mM H_2O_2using TEM, LC3/p62 expression and GFP-LC3 transfection assays.*In vitro* phagocytosis assays found that autophagic dying cells can be efficiently and increasingly engulfed by both professional and non-professional phagocytes over time.

Conclusions: The clearance of autophagic dying ARPE-19 cells can be used as a model for studying both dry and wet type of AMD*in vitro*, as well as for testing future pharmacological agents for treating this disease.

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Are adenosine A3 receptors are required for proper phagocytosis of apoptotic cells?

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Neutrophils migrating towards chemotactic signals release ATP and utilize both P2Y and adenosine A3 receptors (A3Rs) for proper migration. We have shown that macrophages engulfing apoptotic cells also produce adenosine. Since ATP and P2Y receptors were shown to be required also for the migration of macrophages towards the apoptotic cells, we decided to investigate whether adenosine A3 receptors are required for proper phagocytosis of apoptotic cells. Under in vitro conditions, when apoptotic cells are given in excess to macrophages, loss of A3Rs did not affect the efficiency of phagocytosis by A3R null macrophages. However, when apoptosis was induced in vivo in the thymus by intraperitoneal injection of dexamethasone, a delayed clearance was observed by detecting the amount of unengulfed apoptotic cells using annexin V labeling. We are in the process of demonstrating the improper migration of A3R null macrophages towards the apoptotic cells both in vivo and in vitro.

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SECTION 11

Laser scanning cytometric analysis of DNA end-labeling reactions on nuclear halosamples

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The chromosome-long double-stranded genomic DNA molecules harbour single-strand discontinuities, nicks, as revealed by *in situ* enzymatic labelling procedures using e.g. E. coli DNA polymerase (Pol)[1], or terminal deoxynucleotidyl transferase[2] (TdT) enzymes to build labelled nucleotides topre-existing free 3' OHs, or by analyzing DNA samples on denaturing gels[3] that do not degrade misincorporated ribonucleotides. Nicks may be present due to asymmetric topoisomerase II activity, as reported for the TSS of genes upon activation[2], or to topoisomerase I after the "dirtyends" generated by this enzyme are enzymatically processed, or in conjunction with DNA replication. We have been studying the incorporation of labelled nucleotides to sites available for labelling by laser scanning cytometry (LSC) and confocal microscopy (CLSM), using agarose embedded lysed/permeabilized samples of healthy, nonapoptotic Jurkat, MCF7 and MCF7-siTOP1 cells after various enzymatic treatments. We have been investigating labelling also with an RNA/DNA hybrid-specific antibody (S9.6), recognizing R-loops arising in conjunction with transcription and any hybrid longer than 5 bps. LSC allows us to determine the cell-cycle distribution of the traits examined while CLSM helps to reveal the intranuclear pattern of labeling. Our results obtained thus far suggest that (1) topoisomerase I probably doesn't play a significant immediate role in generating the discontinuities detected; (2) labeling of the R-

loops themselves needs to be taken into account when interpreting the data and (3) labelling by all the polymerases used, and to a somewhat less erextent also by the S9.6 antibody, is limited to a topologically isolated subcompartment of the nucleus and exhibit a marked peripheral localization.

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Nucleosome-DNA affinity is highly sensitive to certain H3 modifications and to superhelical twist

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We have developed a sensitive and highthroughput method, applying a laser scanning cytometer (LSC), for the analysis of histone mobility features. Our method offers sensitive means to determine, quantitatively and in a cell-cycle phase specific manner, a major component of global histone mobility: nucleosome-DNA cohesion. After salt or intercalator elicited elution of agarose-embedded isolated nuclei the remaining histone levels are determined by immunofluorescence labeling. Using a panel of modification-specific monoclonal antibodies we have analyzed the elution profiles of H3 histones carrying different epigenetic marks. The elution characteristics of bulk histones were assessed directly in the cell lines expressing GFP-tagged H3.

Significantly steeper profiles (indicating loosely bound histones) could be measured in the case of H3K4me3 and H3K27ac, in contrast with inactive marks like H3K27me2, H3K27me3, H3K9me2, H3K9me3 and other active marks, including H3K9me1, H3K27me1, H3K36me3, H3K4me0, H3K4me1, H3K4me2, H3K9ac, H3K14ac. These data suggest that H3K4me3 and H3K27ac modifications, alone or in combination, have a major role in the basic structural organization of chromatin. This effect is likely to prevail in the absence of reader protein binding to the same modifications, in view of the detection protocol applied. Furthermore, the similar effects of salt and intercalators raise the possibility that superhelical

twist and/or DNA strand extension may play a role in gene regulation via influencing DNA-nucleosome affinity.

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Functional Relevance of DNAJA1 as a Novel Interacting Partner of Human Transglutaminase 2

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Human Transglutaminase 2 (TGM2) is a multifunctional crosslinking enzyme which has a large number of interacting partners contributing to its diverse biological and pathological functions such as cell growth, differentiation, adhesion, migration, apoptosis, neurodegenerative disorders, liver diseases, metastasis and cancer. However the molecular interactions between TGM2 and its interacting partners which govern these processes are largely unknown because of the lack of adequate information regarding these interacting proteins. It is important to identify specific binding partners of TGM2 in different cellular compartments as it may have an impact on the signaling pathways functioning in these cellular processes. Thus the present study aims to identify novel interacting partners of TGM2 and finally explore its functional significance. To achieve this we use NB4 cell line as a model because TGM2 expression is undetectable in wildtype NB4 cell line but upon ATRA (all*trans* retinoic acid) treatment TGM2 expression increases several folds. The differential gene expression analysis of NB4 cell line and TGM2 knocked down NB4 cell line after ATRA treatment revealed that TGM2 was involved in expression of large number of ATRA-regulated genes.
To identify the proteins interacting with TGM2 we employed GST pull down assays and subsequent mass spectrometry analysis. We obtained various novel TGM2 binding candidates namely Tubulin α, Histone H2A and heat shock protein 40 (HSP 40)/DNAJ and in addition to some known interacting partners such as human Glutathione S Transferase (hGST-P1) validating the experimental approach. Since DNAJ and human TGM2 has been reported to be involved in various cancers and regulating the aggregation of proteins in Huntington and Alzheimer disease models we chose DNAJ as one of the candidate protein for further analysis. We performed GST pull down experiment, ELISA, Biacore and co-immunostaining studies with TG2 overexpressing HEK cells and confirmed that TG2 and DNAJ interact with each other and they colocalize in the cytoplasm.
TGM2 has four domains tructure and we use TGM2 domain mutants todetermine the exact binding domain/site of DNAJ. Preliminary ELISA experiments show that core domain of TG2 is the most important domain in this interaction. Biacore experiment will be used to confirm these results.
Preliminary cell adhesion experiments show that overexpression of DNAJ lead to enhanced cell adhesion. In future, we also plan to explore the role of TGM2 and DNAJ interaction on cell migration and additional physiological functions using HEK, HELA and NB4 cell models.

Characterization of cleavage site-mutant capsid proteins of HIV-1

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The HIV-1 capsid protein was found to be an *in vitro* substrate of the protease, and its proteolytic cleavage was predicted to contribute to the disassembly of the viral core in the early stage of infection. Since the virus entering the cells contains the active protease, its activity could be boosted by the acidic pH of the endosomes and the protease-mediated cleavage of viral proteins (including capsid) may be important for the preparation of a functional preintegration complex. Virions containing an inactive protease have no infectivity, suggesting a critical role for the protease in the viral life-cycle. We aimed to study the role of the protease in the early stage of infection by the modification of the previously identified cleavage sites of the capsid protein.

We have introduced several mutations (W23A, A77P, A78V, L189F, L189I, L189P, L190K, L189I/L190K) into a histidine-tagged recombinant capsid protein in order to study the effects of these amino acid substitutions on the proteolytic cleavage efficiency *in vitro*. In the course of our study we also have identified a novel cleavage site in the wild-type capsid protein, and in case of the mutants we observed changes in the processing as was expected based on previous specificity characterization of the HIV-1 protease. Results of circular dichroism spectroscopy measurements showed that the secondary structures of W23A, A77P, L189P, L190K and L189I/L190K mutant proteins remarkably deviated from that of the wild-type. To study the effect of the mutations on the infectivity, we have produced virions containing these mutant capsid proteins using a modified third-generation HIV-1 based vector system. Transduced 293T cells were analyzed for the expression of GFP by FACS and it was observed that both increase and decrease of proteolytic susceptibility reduced the infectivity of the virions. Further studies revealed that mutant proteins with high degree of secondary structure alterations lost their capability to bind the human peptidil-prolyl isomerase cyclophilin A (CypA) protein, while those showing only slight deviations were able to bind CypA as the wild-type. Host-derived CypA has been shown to be incorporated into HIV-1 virions and its incorporation was found to be essential for viral infectivity. The loss of CypA binding ability could contribute to the highly reduced infectivity in the case of the virions carrying capsid proteins with substantially altered structures.

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