

12th Molecular, Cell and Immune Biology Winter Symposium



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

**Debrecen, 10-11 January, 2019
Venue: Theoretical Building**

- 13:50-14:05 Erfaneh Firouzi Niaki:
Effect of DNA targeted drugs on DNA topology
- 14:05-14:20 Rosevalentine Bosire:
Accessibility of DNA in native chromatin to intercalators
- 14:20-14:35 Beáta Boros-Oláh:
Examination of R-loops and chromatin loops with chromatin conformation capture techniques

14:35-15:30 Section 8 (Poster Viewing Session)

Chair: András Mádi

Orsolya Bába: Quantitative analysis of amino acids in tear samples from patients with glaucoma and diabetic retinopathy

Dóra Bencze: Human plasmacytoid and monocyte-derived dendritic cells display distinct metabolic profile upon RIG-1 activation

Nikolett Bertók: Quantitative analysis of tear proteins from patients with diabetic retinopathy with different mass spectrometry-based methods

Dóra Csige: Epitope Mapping of Mouse Monoclonal 885 Antibody on Transglutaminase 2 Enzyme

Noura Faraj: Characterization of individual components of ER α -driven super-enhancers in MCF-7 cells

Hajnalka Emese Halász: Histone acetylation affects monocyte-derived dendritic cell properties

Szilvia Horváth: An attempt to improve anti-transglutaminase 2 autoantibodies measuring kits for the precise prediction of coeliac disease

Zsuzsanna Kolostyak: Transcriptomic analysis of Egr2 transcription factor deficient mouse alveolar macrophages

Kinga Lénárt: Detection of metabolic parameters reveals increased sensitivity of tissue transglutaminase knock-out mice to the α 1-adrenergic agonist phenylephrine

Hung Mai: Immunogenic dendritic cell differentiation from embryonic stem cells with Ikzf1

Vivien Sánta: Enhanced LPS responsiveness of Ccl2, Ccl17, Edn1 and Il12a genes in alternatively polarized murine macrophages

László Sós: Investigation of decreased phagocytic function in mouse macrophages following sodium-palmitate treatment

Beatrix Tolvaj: Characterisation of exogenous transglutaminase 4 expression in AD-293 and PC-3 cells

were carried out on the Orbitrap Fusion mass spectrometer. The results obtained by different methods were compared.

Our results indicate that the combination of label-free quantification and PRM is a good strategy to analyze the quantitative changes of the tear proteome.

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Epitope Mapping of Mouse Monoclonal 885 Antibody on Transglutaminase 2 Enzyme

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The multifunctional, Ca²⁺-dependent cross-linking transglutaminase 2 (TG2) enzyme is the main autoantigen in celiac disease (CD), a chronic immune-mediated enteropathy precipitated by dietary gluten in genetically predisposed individuals. Symptomatic and untreated disease is associated with elevated morbidity and mortality and impaired quality of life. Adherence to a strict, lifelong gluten free diet still remains the single proven and available treatment for CD.

TG2 is a target for autoantibodies in CD found to mainly prefer four conformational epitopes clustered in the N-terminal part of the enzyme. Epitope 1 and 2 appear to be dominant targets across patients. The appearance of typical gastrointestinal symptoms is related to the anti-transglutaminase 2 antibody (anti-TG2) production. Previous studies in our group showed that placenta specimens contained high amounts of anti-TG2 antibodies. Frozen placenta sections were subjected to competition studies with a monoclonal TG2-specific mouse antibody 885 (mAb 885). It selectively interfered with the binding of celiac antibodies to wild type TG2. Such antibodies or similar specific competitors can be useful in functional studies and in exploring whether interference with celiac antibody actions leads to therapeutic benefits.

We addressed to map of the epitope of this mAb 885 on human transglutaminase 2 enzyme. We performed competition ELISA assays on binding of mAb 885 and anti-TG2 antibodies to human TG2. Results showed interference with epitope 2 anti-TG2 binding. Then we analyzed human TG2 mutants to delete the epitope of mAb 885. We also recognized that mAb 885 did not react with mouse TG2. For building up the mAb 885 epitope on mouse TG2 we tested chimera and point-mutated mouse TG2 enzymes.

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Characterization of individual components of ER α -driven super-enhancers in MCF-7 cells

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Approximately 80% of breast cancers are ER-positive. Many investigations have been shown increase in breast cancer risk with lifetime exposure to estradiol through several mechanisms, such as through its binding to the estrogen receptor alpha (ER α).

Whyte and Lovén research teams published at the same time, in 2013, that there are many long regulatory regions in the genome where the outstandingly active enhancers form clusters; these are called super-enhancers (SEs). SEs can be bound by several transcription factors and

moreover, they play an important role in cell identity and fate. However, their role in cancer is still under investigation.

Therefore, we are seeking a genomic characterization of the ER α -driven SEs in the breast cancer-driven MCF-7 cell line, which is the most commonly used *in vitro* model system for the studying the behavior of the ER α . For this, we cultured MCF-7 cells and treated them with estradiol 100 μ M. Then we compared the fold enrichment of three selected SE constituents located at the CUEDC1 gene region over control by implementing chromatin immunoprecipitation followed by qPCR (ChIP-qPCR) technique.

Our study showed that within a SE region, one enhancer, called mother enhancer, had stronger activity than the others, known as daughter enhancers. At the same time, presence of the mother enhancer was detectable before any specific treatment and its activity was significantly higher after activation by estradiol. This is due to the presence of a strong, canonical estrogen response element. This practical result conformed to Bojcsuk, et al results which were gotten using bioinformatics tool. Our study also demonstrated that by increasing the exposure of cells to estradiol, transcription factor ER α interacts much stronger with SEs in breast cancer cells. Hence, this study might give a better understanding and a stronger emphasis on one of ER α molecular mechanisms that are associated with high risk of breast cancer.

HISTONE ACETYLATION AFFECTS MONOCYTE-DERIVED DENDRITIC CELLS PROPERTIES

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Human dendritic cells (DCs) are professional antigen-presenting cells with varied roles in immune responses. Previous studies have shown that monocytes can differentiate *in vitro* to CD1a⁻ and CD1a⁺ DCs in different ratio. This two population of DCs has diverse cytokine secretion and distinct functions in T-cell activation. The expression of CD1a molecule is influenced by many environmental factors which presumably involve epigenetic regulations. Generally, the acetylation of histones results an open and transcriptionally accessible chromatin structure which is regulated by histone acetyltransferase (HAT) and histone deacetylase (HDAC) enzymes. In this study our aim is to observe and understand the effects of histone acetylation on the properties of monocyte-derived DCs (moDCs).

Freshly isolated CD14⁺ monocytes were cultured and differentiated to DCs in AIMV medium. During the differentiation the cells were treated with HAT activator, HAT inhibitor and HDAC inhibitor. Immature DCs were activated at day 3 of culture. Phenotyping of resting and activated moDCs was performed by flow cytometry, the concentration of secreted cytokines was measured by ELISA.

Our results show that the activation of HAT increases CD1a⁺/CD1a⁻ moDC ratio while HAT inhibitor obstructed the expression of CD1a molecule. This kind of inhibition has a disadvantageous effect on DC's viability too. In contrary to our expectations the treatment with HDAC inhibitor results CD1a⁻ DC population in higher proportion. The acetylation modifying agents have effects on activated moDCs too. In case of HAT activator treatment, we noticed changes in CD83, CD86 expression. Interestingly, HDAC inhibitor-treated DCs produced TNF in higher concentration after LPS activation.

Epigenetic modifications, involving the modification of histone acetylation could provide additional useful tools for influencing the immune response and for shaping dendritic cell based therapies in the future. A notably observation of our research is that HAT activator can increase the CD1a⁺ moDCs ratio, although other investigated environmental factors are more likely to induce the appearance of CD1a⁻ phenotype. Inhibiting HDAC resulted the