

**SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
(PHD)**

**TRANSCRIPTIONAL CONTROL OF
TRANSGLUTAMINASE 2 EXPRESSION IN MOUSE
APOPTOTIC THYMOCYTES**

KATALIN DÁNIELNÉ SÁNDOR

SUPERVISOR: PROF. DR. ZSUZSA SZONDY MD, PHD, DSC



UNIVERSITY OF DEBRECEN

DOCTORAL SCHOOL OF DENTAL SCIENCES

DEBRECEN, 2016

TRANSCRIPTIONAL CONTROL OF TRANSGLUTAMINASE 2 EXPRESSION IN MOUSE APOPTOTIC THYMOCYTES

by **KATALIN DÁNIELNÉ SÁNDOR**
MOLECULAR BIOLOGY/BIOCHEMISTRY MSc

SUPERVISOR: **PROF. DR. ZSUZSA SZONDY MD, PHD, DSC**

DOCTORAL SCHOOL OF DENTAL SCIENCES, UNIVERSITY OF DEBRECEN

Head of the Examination Committee: **PROF. DR. KLÁRA MATESZ, MD, PHD, DSC**

Members of the Examination Committee: **PROF. DR. VIKTOR DOMBRÁDI, PHD, DSC**

PROF. DR. FERENC GALLYAS, JR., PHD, DSC

The Examination takes place at Department of Restorative Dentistry, Faculty of Dentistry, University of Debrecen, at 10:30 a.m. on 12th December, 2016.

Head of the Defense Committee: **PROF. DR. KLÁRA MATESZ, MD, PHD, DSC**

Members of the Defense Committee: **DR. DR. VIKTOR DOMBRÁDI, PHD, DSC**

PROF. DR. GALLYAS FERENC, JR., PHD, DSC

Reviewers: **PROF. DR. IMRE BOROS, PHD, DSC**

DR. PÉTER BAY, PHD, DSC

The PhD Defense takes place at the Lecture Hall of Building A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, at 12:30 p.m., on 12th December, 2016.

1. INTRODUCTION

Apoptosis

The term apoptosis was proposed originally by Kerr, Wyllie and Currie in 1972. They observed characteristic microscopic structural changes such as blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation. In addition, the plasma membrane of the apoptotic cell (AC) is radically different from its viable counter-part: it is fundamentally altered in architecture, especially with respect to lipid topology, with loss of phospholipid asymmetry and, in particular, oxidation and redistribution of the phosphatidylserine (PS) from the inner plasma membrane leaflet to the outer.

Mechanisms of Apoptosis

From the mechanistic point of view, apoptosis is a highly complex, sophisticated process. Research in the last two decades show, that there are two main apoptotic pathways in the cells, the extrinsic or death receptor pathway and the intrinsic, or mitochondrial pathway. Later on, it has been shown that the two pathways are interconnected and the regulators of one pathway can affect the other. In addition, a completely different pathway also exists, which involves T-cell triggered cytotoxicity and the so called perforin-, granzyme-dependent execution of the cell. The three apoptotic pathways will converge ultimately on the final, execution pathway, which is initiated by caspase3 leading to DNA fragmentation, degradation of cytoskeletal and nuclear proteins, crosslinking of proteins, formation of apoptotic bodies, expression of ligands for phagocytic cell receptors and finally phagocytic uptake by classical phagocytes.

Apoptosis in the thymic environment

The thymus is the primary lymphoid, multi-lobed organ responsible for the generation of immunocompetent T lymphocytes from bone marrow-derived CD4-CD8- double negative progenitors. This occurs through a series of differentiation and selection steps. The thymic milieu directs these processes via the combinations of available molecules secreted by the dendritic cells, macrophages, endothelial cells, fibroblasts, and thymic epithelial cells (TECs) found in the thymus.

90% of the CD4+CD8+ double positive (DP) thymocytes that are incapable of recognizing self-peptides presented on major histocompatibility complex (MHC) molecules present in the thymus undergo

a default death pathway named “death by neglect”. Thymocytes harbouring a TCR that strongly interacts with MHC molecules containing self-antigens are eliminated by negative selection via activation-induced apoptosis. Thymocytes having TCRs with low-to-moderate avidity for self-peptide/MHC are preserved by positive selection – expressing higher level of Bcl-2, that protect them from apoptosis – and continue to differentiate into CD4+ or CD8+ single positive cells and complete their maturation in the thymic medulla. In the medulla, they will be further exposed to “self-specific” peripheral tissue antigens presented either by medullary TECs as a consequence of TEC expression of the autoimmune regulator transcription factor Aire or by thymic dendritic cells. Those cells that survive this second wave of negative selection will migrate into the periphery and form the peripheral T-cell repertoire. But if a peripheral T-cell does not meet with antigen in periphery, undergo apoptosis soon.

Clearance of apoptotic cells

In vivo, the apoptosis program is completed by phagocytosis, with the majority of apoptotic thymocytes in mice being cleared by the thymic macrophages.

The clearance of apoptotic cells is a multistep process. First, at the early stages of apoptosis, cells that are engaged in the cell death program exhibit so called “find me” signals, which can be recognized by professional phagocytes via their cell surface receptors. These find me signals can be: nucleotide triphosphates (ATP/UTP), lysophosphatidylcholine, CX3CL1 chemokines, which can initiate the chemotaxis of professional phagocytes. Second, with the help of the so called “eat me” signals, deposited on the cell surface, they can be phagocytosed by macrophages. Amongst the “eat me” signals phosphatidylserine is a key molecule. PS can be recognized by the phagocyte-specific Bai1, Tim-4 or Stabilin-2 receptors. In addition, other soluble molecules like thrombospondin-1 (TSP-1), milk-fat-globule-EGF factor-8 (MFG-E8), growth arrest-specific 6 (GAS6) and serum protein S can form bridges between the phosphatidylcholine found on the apoptotic cell surface and the phagocyte receptors, allowing the efficient uptake of apoptotic cells. Finally, the phagocytosed cell/cell parts are digested via the phagolysosomal pathway. As a result, the ingested cell parts can be reused by the phagocyte and the indigestible cell part are excreted.

The clearance of apoptotic cells and cell parts by professional phagocytes represent an evolutionary conserved mechanism, preventing the release of the cytotoxic, or immunogenic cellular components. In the absence of proper clearance, dying cells are able to undergo secondary necrosis, leading to

inflammation, which is a suspected cause of autoimmunity. The most important feature of the phagocytosis of apoptotic cells, that distinguishes it from the phagocytosis of most bacteria or necrotic cells is the release of anti-inflammatory cytokines IL-10, TGF- β , platelet activating factor (PAF), prostaglandin E2 (PGE2) and adenosine (via A2A receptors). Another inherent characteristic of apoptosis is the inhibition of production of pro-inflammatory cytokines (including TNF- α), thus attenuating immune, or autoimmune response. Importantly, it has been shown that failure of apoptotic cell clearance leads to autoimmune disorders such as systemic lupus erythematosus or rheumatoid arthritis, underlying the importance of the clearance of apoptotic cells during normal physiological responses.

Macrophage secreted molecules are inducers of apoptosis in the thymic environment

It has been shown by us and others, that various molecules, mediators are secreted by macrophages in the thymic environment during the engulfment, including transforming growth factor beta (TGF- β), adenosine to prevent proinflammatory cytokine production in an autocrine manner, and retinoids, in an engulfment-dependent manner. These molecules can act on macrophages in an autocrine manner, but importantly, double positive thymocytes express receptors for all the above mentioned molecules. All of these are known as critical for the formation of the pro-apoptotic thymic milieu, that ensures the induction of apoptosis of neglected thymocytes in the absence of TCR signalling.

TGF β

Transforming growth factor- β (TGF- β) signaling plays a critical role in the regulation of cell growth, differentiation, and development in a wide range of biological systems. In general, signaling is initiated with ligand-induced oligomerization of serine/threonine receptor kinases and phosphorylation of the cytoplasmic signaling molecules Smad2 and Smad3 for the TGF- β /activin pathway, or Smad1/5/9 for the bone morphogenetic protein (BMP) pathway. Interestingly, recognition of apoptotic cells via phosphatidyl serine receptors, serving as the first step of apoptotic cell uptake, leads to TGF- β release from macrophages. At the same time, phagocytes produce TGM2, which activates TGF- β . Apoptotic cells and macrophages are also possess TGF- β receptors. In dying thymocytes, TGF- β promotes cell death induced by specific signals and elevates the expression of TGM2, while in macrophages TGF- β increases the phagocytic capacity and reduces the production of pro-inflammatory cytokines. Induction of TGM2 by TGF- β in macrophages results in positive feedback loop leading to more TGF- β production and secretion.

TGF- β suppresses immune responses through two means: inhibiting the function of inflammatory cells and promoting the function of T-reg cells, by inducing Foxp3 expression

Adenosine

Adenosine is a purine nucleoside that, following its release from cells or after being formed extracellularly diffuses to the cell membrane of surrounding cells, where it binds to its receptors. There are four adenosine receptors, all of which are G protein-coupled receptors. The genes for these receptors have been analysed in detail and are designated A1, A2A, A2B, and A3.

Similarly to other Gs-coupled receptors, A2A receptors communicate with the downstream signaling component via the adenylate cyclase enzyme. The activation of the enzyme leads to the increase of the cAMP secondary messenger, which in turn activate the protein kinase A (PKA) enzyme. PKA phosphorylates the cAMP response element binding transcription factor (CREB), leading to its recruitment to cAMP responsive elements in the genome, affecting the expression of the target genes. As an alternative pathway for the cAMP-PKA pathway, upon A2A receptor activation it has been shown that the MAPK signaling can be also active.

The A2A receptor is expressed at high level on the immune cells participating in the organization of the immune response, for example macrophages, dendritic cells, mast cells, neutrophils or even on endothelial cells, showing its presumed, widespread regulatory role on different cell types.

Retinoids

ATRA and 9cRA are physiological ligands for the retinoic acid (RAR) and retinoid X receptors (RXR). These transcription factors are part of the nuclear receptor superfamily and they considered to be ligand-dependent, directly DNA-bound transcriptional regulators. ATRA and 9cRA are able to activate the RAR receptors, while ATRA is not a potent activator of RXR receptors, but it has been shown that its degradation to 9cRA can happen, which does serve as a potent activator of RXR. RXR acts as a promiscuous heterodimerization partner of several NRs, including RAR. The heterodimerization capacity of RXR is needed for the efficient binding of the partner, thus for the appropriate regulation of gene expression. An inherent characteristic feature of RXR heterodimers is whether the dimer can be activated from both sides, named as permissive dimer (e.g. RXR-liver X receptor), or only from the partner's side, known as nonpermissive dimers (e.g. RXR-RAR). The fact that there are multiple isoforms for these receptors, further increase the complexity of the system and explain the pleiotropic physiological action achieved by retinoids.

Transglutaminase 2 (TGM2), a multifunctional enzyme regulating survival and death

Transglutaminase 2 (TGM2) is a unique and most widely expressed member of the transglutaminase enzyme family, and almost all cell types in the body express TGM2 to varying extents; it therefore was previously called “tissue transglutaminase”. Out of its primary enzymatic activity in Ca^{2+} -dependent transamidation of polypeptide chains, it also binds GTP (which blocks transamidation) and may act as a G protein. Moreover, it has been also revealed that TGM2 has a protein disulfide isomerase activity and may also function as a protein kinase. In addition, TGM2 can also function in various biological settings as a protein/protein interaction partner. For example, it has a very high affinity for fibronectin (FN), and together they mediate many adhesion-dependent phenomena, such as cell migration, matrix assembly, and signaling. TGM2 can also bind many integrin receptors, via the extracellular domains of the $\beta 1$ and $\beta 3$ integrin subunits, to maintain cell-extracellular matrix (ECM) interactions independently from fibronectin binding. In cell-matrix interactions, TGM2 can also function as a co-receptor for various isoforms of integrin and FN to form ternary complexes, where all the three proteins successfully interact with each other.

TGM2 appears to be an important enzyme during apoptosis. The main apoptotic function of TGM2 is carried out by its crosslinking activity. The activation of the enzyme leads to highly cross-linked protein polymers in the apoptotic cell compartments, leading to the formation of protein aggregates, which in turn stabilizes and prevents the leakage of harmful intracellular components. Our recent publication reports that overexpression of both the wild type (WT) and the cross-linking mutant of TGM2 (TGM2X) induced apoptosis in Jurkat T cells, but the WT was more effective. Overexpressed WT TGM2 cross-linked RAP1, GTP-GDP dissociation stimulator 1 (RAP1GDS1), an unusual guanine exchange factor acting on various small GTPases. Cross-linked RAP1GDS1 appeared in the ER and induced a yet uncharacterized signaling pathway to promote Ca^{2+} release from the ER via both inositol-triphosphate (IP3) and ryanodine sensitive receptors leading to enhanced mitochondrial Ca^{2+} uptake. Interestingly, TGM2 partly colocalized with mitochondria which contained increased amount of Ca^{2+} . This data indicate that TGM2 might act as a Ca^{2+} sensor in the mitochondria to amplify ER-derived Ca^{2+} signals, and we found a novel mechanism through which TGM2 can contribute to the induction of apoptosis in T cells.

TGM2 can both facilitate and prevent apoptosis. It seems that depending on the actual apoptosis pathway and the cell type, its activities can be opposing. During thymocyte apoptosis, it has rather proapoptotic activity.

Macrophage secreted molecules are regulators of Tgm2 expression in dying thymocytes

TGM2 has been known for a long time to be associated with the *in vivo* apoptosis program of various cell types, including T cells. TGM2 expression is induced in thymocytes dying *in vivo* following exposure to various apoptotic signals, and TGM2 also appears in the dying T lymphocytes of HIV-infected individuals. While, TGM2 is strongly induced in dying thymocytes *in vivo*, no induction of TGM2 was observed, when thymocytes were induced to die by the same stimuli *in vitro* indicating that signals arriving from the tissue environment contribute to the *in vivo* induction of the enzyme in apoptotic thymocytes. Later on, studies from our laboratory have demonstrated that in dying thymocytes the expression of TGM2 is induced by signals derived from engulfing macrophages, such as retinoids, TGF- β and adenosine, the latter triggering the adenylate cyclase signaling pathway.

A promoter centric view on the transcriptional regulation of Tgm2

TGM2 is mainly regulated at the transcriptional level and it has been shown that the human TGM2 promoter is responsive to several signaling molecules. Retinoids are the most well-known activators of TGM2 via a tripartite response element in the human TGM2 promoter located approximately 1.7 kb upstream relative to the transcription start site. The promoter also possesses a TGF- β located approximately 0.9 kb upstream of transcription start site. These studies identified the exact elements for retinoid and TGF- β signaling in the promoter region of the gene, further supporting the direct regulation of TGM2 by these signaling pathways.

Mapping the location and features of intergenic enhancer elements

In metazoans, *cis*-acting elements are dispersed and can be located far away from their target genes. Enhancers are crucial regulators of gene expression and it has been shown in one of the earliest studies that their mutations may result in congenital diseases.

Enhancers are DNA sequences with the capability to recruit various types of transcription factors for the interaction with the mediator complex, as well as with the members of the (pre-)initiation complex. Through chromatin looping mechanisms, the complexes assembled on the DNA can facilitate RNA-polymerase II (RNAPII) loading to the promoter, thus contributing to the initiation of gene transcription. It has been also shown that transcription factors, acting on enhancers are able to remodel the surrounding chromatin structure to establish NFRs (Nucleosome-free regions) by recruiting ATP-dependent chromatin remodelers, hence facilitating transcription factor binding, transcription initiation

and elongation. Genome-wide studies mapping nucleosome occupancy indicate that, histone replacement is more enhanced at cis-regulatory elements than at other genomic locations not harbouring enhancer-like properties. Active promoters/TSSs are barely occupied by nucleosomes, thus they are nucleosome-free. High-resolution co-activator CREB-binding protein (CBP) and P300 ChIP-seq experiments provided further insights into the chromatin signatures of enhancers, showing that P300 and CBP are general markers of enhancers. Certain histone modifications also take part in cis-element function and it has been shown that active promoters are characterized by the presence of RNAPII and TBP-associated factor 1 (TAF1), marked by NFRs surrounded by trimethylated histone H3 at lysine 4 (H3K4me3), acetylated H3 (H3ac) and TFIID. As previously mentioned P300 is one of the well-documented and accepted active enhancer marks along with the combination of H3K27ac and H3K4me1 or H3K4me2. These observations were evidenced in different cellular model systems resulting in the identification of enhancer landscapes in certain cell types. The discovery of eRNAs added another layer to enhancer features and it turned out that this can be one of the most reliable marker that the enhancer actively participates in gene regulation.

Based on advanced sequencing technologies, in 2012 the ENCODE (Encyclopaedia of DNA elements) Consortium has released genome-wide datasets in the form of 30 papers. These studies aimed to identify the cis-acting element repertoire of the human genome and also several mouse cell lines. In addition, they provided an enormous amount of genome-wide data also from various mouse tissues. These datasets are of great help to identify the putative enhancer elements repertoire of a given gene, knowing the basic features of active enhancers and how genome topology is shaped via the above mentioned organizers of 3D chromatin structure. We have successfully exploited these datasets from mouse thymus in order to predict the functional enhancer repertoire of the apoptotic regulator gene *Tgm2*.

2. AIMS OF THE STUDY

Previously our research group has shown that TGM2 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 transforming growth factor beta (TGF- β) released by engulfing macrophages are involved in this process. Since adenosine is also produced by macrophages during engulfment of apoptotic cells, we decided to

- investigate the possible involvement of adenosine in the TGM2 induction in apoptotic thymocytes.
- determine the mechanism of adenosine production during engulfment.

Due to its ubiquitous appearance and multiple functions, the expression of the Tgm2 gene must be tightly controlled. The *in vivo* appearance of TGM2 protein in dying thymocytes seems to be regulated at the level of transcription. Thus we decided to

- identify the potential enhancer elements of Tgm2 in thymocytes
- and characterize them by measuring the
 - eRNA production
 - binding of transcription factors (RXR, RAR, CREB, SMAD4)
 - recruitment of coactivators (P300, CBP) and H3K27ac histone marker

3. MATERIALS AND METHODS

Experimental animals

Most of the experiments were carried out with 4-wk-old C57Bl/6 mice. In some experiments A3R deficient mice generated on a C57Bl/6 background developed and provided to us by the Merck Co., NJ, USA, pannexin-1 null mice generated a C57Bl/6 background and A2AR deficient mice generated on an FVB background were also used. Mice were maintained in specific pathogen-free condition in the Central Animal Facility of our university, and all animal experiments were approved by the Animal Care and Use Committee of University of Debrecen (DEMÁB).

Thymocyte cultures

Isolated thymocytes (1×10^7 /ml) were cultured in RPMI medium 1640 supplemented with 10% charcoal stripped FBS, 2 mM glutamine, 1 mM Na pyruvate, 100 U/ml penicillin and 100 mg/ml streptomycin at 37°C in 5% CO₂. Cells were exposed to 5 µM NECA (5'-N-ethylcarboxamidoadenosine), a non-metabolisable adenosine analogue, to 1 µM CGS21680, an A2AR agonist (Tocris Bioscience, Eching, Germany), to 1 µM forskolin, an adenylyl cyclase activator, to 100 µM Rp-cAMPS triethylamine, a competitor of endogenous cAMP or to 100 µM db-cAMP, a cell permeable cAMP analogue, 1 µM AM580, an RAR agonist, 100 nM LG268, an RXR agonist alone, or in combination with 5 ng/ml recombinant human TGF-β1 (AbD Serotec, Kidlington, UK) and 0.3 µM 9-cis retinoic acid (9cRA) (Tocris Bioscience, Eching, Germany) for the indicated time periods.

Induction of thymic apoptosis in vivo

4-wk-old mice were injected intraperitoneally with 0.3 mg dexamethasone-acetate - dissolved in DMSO/physiological saline to induce thymic apoptosis. Controls received the same dose of DMSO/physiological saline. Thymuses were removed at the indicated time points. Thymic apoptosis was evaluated by measuring the change in the amount of thymic weight.

Macrophage isolation and culturing

Macrophages were obtained by peritoneal lavage with sterile physiological saline from 3- mo-old C57Bl/6 mice. Cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 2 mM glu-

tamine, 1 mM Na-pyruvate, 50 μ M 2-mercaptoethanol, 100 U/ml penicillin and 100 μ g/ml streptomycin at 37°C in 5% CO₂ for 2 d before use. After 3-4 h incubation, the non-adherent cells were washed away. Before the experiments the cells were cultured for 2 d replacing media daily.

Western Blot analysis

Collected thymuses were homogenized in ice-cold lysis buffer containing 0.5% Triton X-100. Protein concentration of each samples were diluted to 2 mg/ml, then the samples were boiled with an equal volume of Laemmli buffer. 40 μ g protein was run on an polyacrylamide gel, and blotted onto polyvinylidene difluoride membranes using the Bio-Rad electrophoresis and transfer system. The free binding sites of membranes were blocked with 5% non fat dry milk powder in 20 mM Tris, 0.1 M NaCl buffer with 0.1% Tween overnight at 4 °C. 2 μ g anti-TG2 antibodies (Santa Cruz Biotechnology) were added to the membranes. Equal loading of protein was demonstrated with probing the membranes with anti-TG2 and β -actin (Tocris Bioscience, Eching, Germany) antibody.

Determination of cytokine production

Peritoneal macrophages were plated onto 24-well plates at a density of 1×10^6 cells / well. To determine cytokine production by macrophages exposed to apoptotic cells, macrophages were exposed to apoptotic cells for 1 h in the presence or absence of the ecto-5'-nucleotidase inhibitor methylene adenine-5'-diphosphate and NECA. Apoptotic cells than were washed away, the compounds were re-added and the macrophages were cultured for an additional five hrs. At the end of culture cell culture media were analyzed by Mouse Cytokine Array (Proteome Profile Array from R&D Systems). The pixel density in each spot of the array was determined by Image J software. Alternatively, cytokine-induced neutrophil-attracting chemokine (KC), and macrophage inflammatory protein-2 (MIP-2) cytokine levels were measured with R&D Systems ELISA kits.

ATP determination

Apoptotic cells were prepared from 4-wk-old wild-type or pannexin-1 null mice. The isolated thymocytes were cultured for 24 hrs. (10^7 cells/ml) in RPMI 1640 medium supplemented with penicillin/streptomycin in the absence of serum. The culture medium was then collected for ATP determination by the ATPlite Luminescence ATP Detection Assay System (PerkinElmer) according to the manufacturer's suggestions.

Real-Time Quantitative PCR (qRT-PCR) for detecting Tgm2 and Rprd1b mRNA levels

RNA was isolated with Trizol Reagent according to the manufacturer's guidelines. For mRNA measurements, High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Budapest, Hungary) was used and transcript quantification was performed by Quantitative Real-Time PCR reaction using SYBR green dye (gb SG PCR Master Mix, Generi Biotech, Czech Republic). For enhancer RNA measurements, RNA was isolated with the same Trizol based method, but samples were digested with DNaseI enzyme for 1 hour at 37°C. RNA was then reverse transcribed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Budapest, Hungary) and quantification was performed with LightCycler® 480 SYBR Green I Master (Roche, USA). The following PCR program was used for enhancer RNA and mRNA detection: 95°C-3 min; 95°C-30 sec; 60°C-1 min. The last two steps were repeated 40 times. Transcript levels were normalized to Ppia. For enhancer RNA measurements, we designed eRNA specific primer pairs targeting the enhancer sequences upstream to their DNase hypersensitive sites, but on the enriched histone modifications. We used primer3 software to design the eRNA specific primers with standard conditions except the followings: amplicon length: 90-150bp; Tm: 59-60°C.

Chromatin immunoprecipitation followed by qPCR

Cells were crosslinked with Di(N-succinimidyl) glutarate (ProteoChem, USA) for 30 minutes and then with formaldehyde for 10 min. Crosslinking reaction was terminated by the addition of glycine. Chromatin was sonicated with Diagenode Bioruptor to generate 200-1000 bp fragments. Immunoprecipitation was performed with antibodies against pre-immune IgG (Millipore, 12-370), RXR (sc-774), P300 (sc-585), CBP (sc-369X), CREB1 (ab31387), SMAD4 (sc-7154X), H3K27ac (ab4729) from Merck (Budapest, Hungary), Santa Cruz Biotechnology (Dallas, USA) or Abcam (Cambridge, UK). Chromatin antibody complexes were precipitated with Protein A-coated paramagnetic beads (Life technologies, USA). After 6 washing steps complexes were eluted and reverse crosslinked. DNA fragments were column purified (Qiagen, MinElute). DNA was diluted and used for qPCR analysis.

Chromosome conformation capture (3C)

Thymocytes were isolated and cross-linked using 2% formaldehyde for 10 min. Cross-linking reaction was quenched with glycine and nuclei were isolated. Chromatin was digested with HindIII restriction enzyme overnight at 37°C. On the following day digested chromatin was ligated in the presence of T4

DNA ligase (50U) at 16°C for 16 h. Ligated DNA fragments were isolated by phenol:chloroform extraction. PCR reactions were performed by using approximately 100 ng 3°C template.

Statistical analysis

All data are presented as means \pm SD. We made three biological replicates in all the experiments and we performed paired (two tailed) t tests. Results were considered significant with $p < 0.05$.

4. RESULTS

Activation of the adenosine A2A receptor contributes to the upregulation of TGM2 in thymocytes dying in vitro

Since our previous studies indicated that macrophage-derived TGF- β and retinoids are involved in the regulation of TGM2 expression in dying thymocytes, we wanted to test whether adenosine, which is also produced during engulfment of apoptotic cells, could also affect TGM2 expression in thymocytes. Adenosine can be efficiently metabolized by adenosine deaminase of thymocytes, we applied NECA, a non-metabolisable analogue of adenosine known to act on all subtypes of adenosine receptors. For measuring TGM2 mRNA expression, thymocytes were exposed to NECA for 6 hours, as previous studies have indicated that the mRNA expression of TGM2 peaks at this time point in thymocytes. Administration of NECA resulted in a 2 fold induction in the mRNA expression of TGM2 in wild type thymocytes implying that adenosine might be involved in the regulation of TGM2 expression.

Thymocytes have been shown to express adenosine A2A (A2AR) and A3 (A3R) receptors. In order to decide which adenosine receptors are involved in the adenosine-mediated induction of TGM2 at the mRNA level, we exposed thymocytes isolated from A2AR or A3R null mice to NECA. NECA could significantly enhance the mRNA expression of TGM2 in A3R null thymocytes, however it had no effect in A2AR null cells on the expression of TGM2 at the mRNA level. These data indicate that adenosine mediates its effects on TGM2 mRNA expression via adenosine A2A receptors.

Next, we focused on the effect of selective A2AR activation on TGM2 mRNA expression alone or in combination with TGF- β and retinoic acids (RAs) by using CGS21680, a synthetic A2AR agonist. Since in our previous studies indicated that 9-cis retinoic acid (9cRA) is the most effective retinoid in inducing TGM2 at the mRNA level, 9cRA was selected to be used in this study. 9cRA and TGF- β were applied in a concentration that was shown to be effective to affect apoptosis of thymocytes, while CGS21680 was administered at 1 μ M concentration, because we have shown previously by using A2AR null cells that CGS21680 administered at this concentration selectively activates A2ARs. Exposure to TGF- β 9cRA or CGS21680 alone all increased the mRNA expression of TGM2 in cultured thymocytes, while no increase was found in the endogenous mRNA expression of TGM2 during the 6h culture period. Among the compounds tested alone, 9cRA was the most effective in inducing the

mRNA expression of TGM2 resulting in a robust 5 fold increase as compared to the DMSO-treated control.

Activation of A2ARs had no significant effect on TGF- β -induced TGM2 expression at the mRNA level, but significantly enhanced the 9cRA-induced TGM2 mRNA expression. As we reported previously TGF- β could also significantly enhance the 9cRA-induced TGM2 expression. However, the most significant (about 20 fold) induction was found, if the three compounds were applied together. While the induction of TG2 mRNA could be well detected in thymocytes dying *in vitro* exposed to the above compounds, the TG2 protein remained still invisible, even if we performed the experiments in the presence of the caspase 3 inhibitor Ac-DECD-CHO to prevent a possible degradation by caspase 3. Altogether these data indicate that *in vitro* retinoids, adenosine and TGF- β , compounds all known to be produced by engulfing macrophages, can work together to significantly induce the levels of TGM2 in dying thymocytes.

Adenosine A2A receptors affect the expression of TGM2 in thymocytes via the adenylate cyclase pathway

Previous studies from our laboratory have shown that though A2ARs can signal through both the adenylate cyclase and the phospholipase C system in other cell types, A2ARs in mouse thymocytes specifically activate the adenylate cyclase signaling pathway. Thus we administered 100 μ M Rp-cAMPS triethylamine, a specific membrane-permeable competitor of intracellular cAMP, to test whether adenylate cyclase signaling indeed contributes to TGM2 induction. As shown in Figure 2A, in the presence of Rp-cAMPS triethylamine activation of A2ARs by CGS21680 alone or in combination could not enhance TGM2 mRNA expression in thymocytes.

Then we further tested whether activation of the adenylate cyclase pathway alone is sufficient to induce the expression of TGM2 at the mRNA level in mouse thymocytes. As shown in Figure 2B and C, exposure of thymocytes to forskolin, an adenylate cyclase activator, or to dibutyryl-cAMP, a membrane permeable analogue of cAMP, alone or together with 9cRA or 9cRA and TGF- β resulted in enhanced TGM2 mRNA expression. Altogether these data indicate that compounds increasing intracellular cAMP levels can contribute to the TGM2 mRNA expression in mouse thymocytes, and A2AR signaling enhances the expression of TGM2 by triggering the adenylate cyclase pathway.

Specific activation of the adenosine A2A receptor contributes to the upregulation of TGM2 in dying thymocytes in vivo

In the following experiments we wanted to test the idea whether adenosine produced in the context of the in vivo apopto-phagocytosis program of thymocytes could also contribute to the upregulation of TGM2. For this purpose apoptosis was induced by intraperitoneal injection of dexamethasone in the thymus of both A2AR null mice and their wild type counterparts. Dexamethasone is a powerful apoptosis inducer of thymocytes, and if adenosine is produced under in vivo conditions and contributes to the in vivo upregulation of TGM2, we should detect an altered TGM2 expression in A2AR null mice. Indeed, in the thymus of A2AR null mice, the dexamethasone-induced TGM2 expression was significantly attenuated at both mRNA and protein levels. The failure of inducing TGM2 fully in the thymus of A2AR null mice was not related to a failure of thymic apoptosis induction, as no significant difference in the loss of thymic weight was detected between the two mouse strains. This is in line with our previous results, which demonstrated that A2AR null thymocytes show similar apoptotic sensitivity to dexamethasone, as their wild type counterparts. Altogether these data indicate that A2AR signaling is the third pathway that contributes to the upregulation of TGM2 in dying thymocytes under in vivo conditions.

Our data provide evidence that the A2AR signaling pathway via the activation of adenylate cyclase, contributes to the expression of *Tgm2* in dying thymocytes. To date there are at least three different signaling pathways that can regulate the expression of *Tgm2* at the mRNA level, but how these act together and what kind of intergenic regulatory elements are available serving as molecular beacons for these around the gene, remained completely elusive.

In our next study, we aimed to determine the functional enhancer element repertoire through which the described activator molecules and their downstream transcription factors act in order to regulate the level of *Tgm2* in dying thymocytes.

The combination of DNase-seq and ChIP-seq allows the identification of the putative enhancer set of *Tgm2*

In order to identify the putative enhancer elements, which might play a role in the regulation of *Tgm2* in mouse thymocytes, we decided to use several mouse thymus derived datasets generated by the ENCODE Consortium. More specifically, we utilized DNase-seq data, ChIP-seq datasets for H3K4me1, H3K4me3, and H3K27ac for enhancer identification and CTCF ChIP-seq to determine the

putative gene domain for Tgm2 in which the regulation might take place. As a very first step, we tried to exploit the recently described feature about the motif orientation of CTCF and its effect on long-range chromatin looping and demarcating functionally different genomic regions. Tandem orientation of the CTCF motifs has been proposed to play role in gene regulation by allowing the enhancers to find their targets, while convergent motifs are more likely to be implicated in the formation of higher-order chromatin structure. We identified three high affinity CTCF peaks flanking a 157kb wide genomic region around Tgm2. Analysis of the orientation of the motifs showed one convergent motif pair located at the borders of the 157kb genomic area, while the third, transcription start site (TSS) specific motif constitutes a tandem motif pair with the upstream CTCF border. These data suggest that the regulation of the gene might take place in a genomic region spanning 157 kb pairs.

While convergent motifs are likely to function in establishing topologically associated domain borders, there are still many exceptions in which case potential enhancers could be overlooked. To avoid this possibility, we overlapped the interaction profile of a genomic loci of ~800kbp containing the Tgm2 locus with the available CTCF ChIP-seq and DNase-seq from the CH12.LX mouse B lymphoblast cells and the same datasets from thymus along with the histone modification ChIP-seq profiles. We found that interactions between the two proposed boundaries are clearly enriched suggesting that the regulation of Tgm2 most probably takes place in this genomic unit and the enhancers do not communicate with the neighboring genes. In addition, we also measured the mRNA expression of the neighboring gene (Rprd1b), which seems to be active based on the H3K4me3 histone modification present at the transcription start site of the gene. However, we could not detect any difference in its expression level in the presence of those compounds which affected the expression Tgm2.

Next we took advantage of the available DNase-seq dataset and identified several hypersensitive sites around the Tgm2 gene located between the tandem CTCF sites, and an additional one located between the TSS-specific and the downstream CTCF site, which show convergent motif orientation (Figure). Since integration of DNase-seq with ChIP-seq against H3K4me3, H3K4me1 and H3K27ac proved to be useful in the identification of putative functional enhancers, we applied this approach and found six (+30kb, TSS, -7.9kb, -13kb, -20kb, -28kb) hypersensitive regions which showed enrichment for H3K4me3 and for H3K4me1. H3K4me3 is a hallmark of actively transcribed protein coding gene promoters, but can be detected occasionally together with enhancers due to a strong enhancer/promoter interaction. H3K4me1 is associated with most of the accessible regulatory elements in the genome and represents active, poised or primed enhancers depending on the presence of other histone

modifications. Out of the six elements, only two (-13kb and -20kb) showed enrichment for H3K27ac, which is a widely accepted functionally active enhancer marker.

Using the ENCODE generated genome-wide datasets from mouse thymus we have identified five intergenic enhancers for detailed analysis excluding the TSS/promoter region, which has been studied thoroughly.

Whole thymus tissue derived acetylation patterns correlate with the acetylation pattern of in vitro cultured thymocytes

The thymus is composed of various types of cells. Although thymocytes constitute the majority of cells in the thymus, one should be careful of using whole thymus for enhancer mapping, because other cell types, such as macrophages, dendritic cells and epithelial cells are also present. Therefore, signals are not reliably originated from the cell type of interest. To make sure that we have a pure population of thymocytes we isolated them from the mouse thymus to check whether the selected putative enhancers of Tgm2 in these cells show a similar pattern of H3K27ac.

ChIP-qPCR measurements against CBP, P300 and H3K27ac from in vitro cultured mouse thymocytes are shown on the indicated intergenic regulatory elements. As a control, we also performed the same ChIP-qPCRs on the neuron specific Prmt8 gene -1.7 kb region, which in the thymic genome appears as a non-DNase hypersensitive region (non-DHS). Data represent mean \pm SD of triplicate measurements.

Enhancer RNA measurements identify functional enhancers for the Tgm2 gene

Previous studies from our laboratory have shown that in thymocytes Tgm2 expression is regulated by external signals derived from engulfing macrophages, such as retinoids, TGF- β and adenosine, the latter activating adenosine A2A receptors and the consequent adenylyl cyclase pathway. Among these signals 9cRA was the most effective in inducing Tgm2 mRNA levels, resulting in a 5 fold induction. Activation of both the adenylyl cyclase and the TGF- β signaling pathway synergized with the retinoid signaling pathway in inducing Tgm2 mRNA levels.

To determine whether the putative enhancers respond to these signals, we decided to measure the amount of eRNAs transcribed from them. To detect the response of the individual enhancers, primary

thymocytes cultured *in vitro* were exposed for 1 h to 0.3 μM 9cRA, a ligand of the nuclear retinoic acid receptors (RAR/RXR), to 5 ng/ml rTGF- β leading to trimerization of SMAD4 with phosphorylated SMAD2 or 3 and its nuclear transport, or to 100 μM dbcAMP, a membrane permeable analogue of cAMP, resulting in the activation of protein kinase A and in subsequent phosphorylation of CREB. However, phosphorylation and activation of CREB can occur also via activation of both the TGF- β signaling pathway, which activates protein kinase A in a cAMP-independent manner leading to the release of its regulatory subunit and the retinoid signaling pathway, which can lead to CREB phosphorylation in a protein kinase A-dependent and independent manner.

Interestingly, all of the enhancers showed elevated eRNA expression in response to 9-cRA, but only two of them, located -13kb and -20kb, appeared to be regulated also by TGF- β or dbcAMP. In addition, these two enhancers showed clear differences in their retinoid response showing a much robust eRNA upregulation as compared to the +30kb, -7.9kb and -28kb enhancers.

Our knowledge about these signaling pathways allowed the investigation of the binding of the signal specific transcription factors on these enhancers. We applied ChIP-qPCR to measure the enrichment of CREB, SMAD4, RAR and RXR in the presence of their activators on the identified enhancer elements. Exposure to dbcAMP efficiently recruited CREB to each enhancer elements, but the occupancy of CREB appeared to be the highest when we tested its binding on enhancers located at -13kb and -20kb. In accordance with reports, which demonstrated CREB activation in the retinoid and TGF- β signaling pathways, enhanced CREB binding could be detected also following exposure to 9cRA or rTGF- β , but again the occupancy of CREB appeared to be the highest when we tested its binding on the enhancers located at -13kb and -20kb. Similarly, when we assessed the binding of SMAD4 in the presence of rTGF- β , we found a similar pattern of dynamic recruitment on the investigated enhancers with the exception of the -7.9kb and -28kb enhancers which did not show any enrichment. ChIP-qPCR experiments performed against RAR or RXR in the presence of 9cRA did not show robust recruitment on the investigated enhancers. Still, an enhanced binding of RAR and RXR, as a 9cRA response, was found on all the investigated enhancers. In line with the eRNA measurements, these results indicate that the majority of RARs and RXRs are already bound to all of the investigated sites even in the absence of the externally added agonist, as it was previously shown by others.

Our results demonstrate that the strategy developed by us proved to be efficient in the identification of signal selective enhancers. By measuring the synthesized eRNA species from individual enhancers and combining the results with transcription factor binding data, we identified five signal selective enhancers of Tgm2 in mouse thymocytes.

The signaling pathway induced by TGF- β selectively potentiates the retinoid signaling on Tgm2 associated enhancer elements

Recently, it has been shown by our group that the adenylate cyclase and TGF- β mediated signaling synergize with the retinoid signaling on TGM2 leading to a more pronounced induction of TGM2 at protein level. In line with the protein data, we found by using qRT-PCR analysis that administration of TGF- β could significantly increase the 9cRA-induced mRNA expression of Tgm2. Based on these results we decided to test, whether the activation of TGF- β signaling could potentiate the retinoid-mediated signaling pathways in the context of Tgm2 regulation. To identify those enhancers on which the collaboration between the two signaling pathways might take place, we determined enhancer specific eRNA levels in the presence of various signal combinations. As shown in Figure 3B, enhancer elements located at +30kb and at -28kb did not show any sign of collaboration in agreement with our previous results, which indicated that on these elements no significant TGF- β -induced SMAD4 binding was found. On the other hand, we could detect significantly increased eRNA expression on the -7.9kb, -13kb and -20kb enhancers if both signals were present, indicating that the TGF- β and the retinoid pathways crosstalk on these enhancers. To analyze this crosstalk in more detail, we performed ChIP-qPCR experiments using RXR specific antibodies. While 9-cRA alone resulted only in a slight increase in the RXR recruitment, a significant increase in the recruitment of RXR to the enhancers located -7.9kb, -13kb and -20kb could be detected, when TGF- β was also administered. Interestingly, in the presence of both signals the SMAD4 recruitment increased also. Moreover, the recruited P300 and CBP showed also elevated levels on these enhancer elements in response to TGF- β /9cRA co-treatment as compared to 9cis-RA alone. These results suggest that in the presence of both signals the accessibility of these sites increases for both transcription factors, which ultimately leads to an increased coactivator binding and more efficient transcription. Interestingly, in the case of the -7.9 kb enhancer the interaction existed between the two pathways despite the lack of significant binding of SMAD4 to this enhancer in the presence of TGF- β alone.

The adenylate cyclase pathway also selectively potentiates retinoid signaling on Tgm2 associated enhancer elements

Since previous studies indicated that the adenylate cyclase pathway also enhances TGM2 protein levels in the thymocytes *in vivo*, we determined Tgm2 mRNA levels in the presence of dbcAMP as well. Exposure to dbcAMP alone enhanced the steady state levels of Tgm2 mRNA, and when dbcAMP was applied together with 9cRA, it synergized with retinoid signaling in regulating Tgm2 mRNA levels,

though less efficiently as TGF- β did. Next by measuring eRNA levels on the various enhancers, we examined whether the two signaling pathways synergize also in the context of Tgm2 regulation. Enhancer elements located at +30kb, -7.9kb and -28kb did not show any sign of collaboration in agreement with our previous results, which indicated that on these elements no significant dbcAMP-induced CREB binding was found. On the other hand, we could detect significantly increased eRNA expression on the -13kb and -20kb enhancers if both signals were present, indicating that the adenylate cyclase and the retinoid signaling pathways crosstalk on these enhancers.

To analyze this crosstalk in more detail, we performed similar ChIP-qPCR experiments for transcription factor binding, as we did for studying the TGF- β /retinoid signaling crosstalk. However, exposure to dbcAMP did not enhance the 9cRA-induced RXR recruitment to any of the enhancers. The recruitment of CREB, on the other hand, on most enhancers was higher in the presence of the two signals, than in the presence of 9cRA alone, but not higher than in the presence of dbcAMP alone. Thus, while in the case of the TGF- β /retinoid crosstalk we detected increased enhancer accessibility for RXR, when the two signals were present together, no such an enhancement for RXR binding was detected in the adenylate cyclase/retinoid signaling crosstalk. In addition, despite the fact that more CREB was recruited to most of the enhancers in the presence of the two signals, neither more CBP, nor more P300 coactivator binding were detected in the presence of dbcAMP, as compared to 9cRA alone.

Chromosome conformation capture experiments reveal interactions between the -20kb enhancer and the Tgm2 gene

As a control for our approach, we performed chromosome conformation capture experiments to prove that the enhancers, we identified, interact with Tgm2 gene. For this purpose we selected the +11kb and the +30 kb (-3' UTR) regions of the gene, which are DNase hypersensitive and as a control the +13kb region, which is a lowly accessible region. The limitation of the method related to the HindIII restriction sites allowed us to investigate the interaction of these regions with the potential -20kb enhancer. We were able to demonstrate the interaction between the enhancer located -20kb and the intronic region of the gene. Interestingly, we also found an interaction with the 3' UTR of the gene, where we also identified a DNase hypersensitive site, but could not show the interaction with the intervening sequence (+13kb region). Importantly, none of these contacts appeared to show induced interactions in the presence of the applied agonists, indicating that these are stable, pre-formed loops between the enhancer and the gene. We have previously reported that the both the clearance of apoptotic cells clearance, and the LPS-induced pro-inflammatory cytokine production is disturbed in

TG2^{-/-} macrophages. Hence, the age-dependent autoimmunity of TG2 null mice is probably caused by these defective processes. Since the activation of TGF- β – which not only mediates the anti-inflammatory effects of ACs but also takes a part in the resolution of LPS-induced inflammatory responses – is defective in the absence of TG2, it is very likely that the lack of TGF- β is responsible for the enhanced inflammatory responses of TG2 null macrophages. With our present study we wanted to test this hypothesis. Our questions were:

5. SUMMARY

Apoptosis is a physiological process, critical for the elimination of cell types that are not needed or represent threat to the body. Day by day, billions of cells undergo apoptosis and cleared by professional phagocytes, without any inflammatory response. An important component of the cell death program is transglutaminase 2 (TGM2), which is a multifunctional enzyme, critical for proper apoptotic cell recognition and clearance and it also prevents the release of harmful, cytotoxic cell contents via its protein crosslinking activities. The enzyme is highly upregulated during T cell apoptosis in the thymus. The mouse thymus is very active apoptotically, because above 90% of the T cells die in the thymus during their maturation and selection. Based on this, mouse thymus-derived apoptotic thymocytes represent an excellent model system for studying the regulation of the enzyme by the molecules present in the thymic milieu *in vivo*.

In the first part of my thesis, I present how thymus-specific molecules, known to be secreted by macrophages, for example TGF- β , adenosine and retinoids contribute to the upregulation of TGM2 at the gene expression level. We found that adenosine work in concert with TGF- β and retinoic acid to significantly enhance the level of Tgm2 in dying thymocytes *in vitro*. Moreover, adenosine acts via the adenosine A2A receptor – adenylyl cyclase pathway and contributes to the upregulation of TGM2 *in vivo*. We also demonstrate that adenosine is produced extracellularly during the clearance of apoptotic thymocytes, partly from adenine nucleotides released by pannexin-1 channels, revealing a novel cross-talk between macrophages and apoptotic cells.

In the second part of my work, I present how thymus-specific mediators regulate the expression of Tgm2 using different intergenic regulator elements. Due to technical limitation, so far only the promoter region of the gene has been investigated in detail with the mostly artificial reporter assays. The role of intergenic regulatory elements remained elusive in the context of TGM2 in thymocytes. We overcome this limitation by exploiting genome-wide datasets of ChIP-seq and DNase-seq from the ENCODE consortium. We revealed the putative regulatory elements of the gene by the combination of these technologies and identified the ones that might be functionally important for gene regulation, measuring enhancer-specific RNA transcripts (eRNAs) by mimicking the activation of TGF- β , retinoic acid and adenosine triggered signaling pathways. Our study describes a novel strategy to identify and characterize the signal-specific functional enhancer set of a gene by integrating genome-wide datasets and measuring the production of eRNA molecules.



UNIVERSITY OF DEBRECEN
UNIVERSITY AND NATIONAL LIBRARY



Candidate: Katalin Sándor
Neptun ID: LMPHZ6
Doctoral School: Doctoral School of Dental Sciences

Registry number: DEENK/142/2016.PL
Subject: Ph.D. List of Publications

List of publications related to the dissertation

1. **Sándor, K.**, Dániel, B., Kiss, B., Kovács, F., Szondy, Z.: Transcriptional control of transglutaminase 2 expression in mouse apoptotic thymocytes.
Biochim. Biophys. Acta, Gene Reg. Mechan. "Accepted by Publisher" (2016)
IF:6.332 (2014)
2. **Sándor, K.**, Pallai, A., Düró, E., Legendre, P., Couillin, I., Sággy, T., Szondy, Z.: Adenosine produced from adenine nucleotides through an interaction between apoptotic cells and engulfing macrophages contributes to the appearance of transglutaminase 2 in dying thymocytes.
Amino Acids. Epub ahead of print (2016)
DOI: <http://dx.doi.org/10.1007/s00726-016-2257-5>
IF:3.293 (2014)





UNIVERSITY OF DEBRECEN
UNIVERSITY AND NATIONAL LIBRARY



List of other publications

3. Hsieh, Y., Liu, G., Lee, Y., Yang, J., **Sándor, K.**, Sarang, Z., Bononi, A., Pinton, P., Tretter, L., Szondy, Z., Tsay, G.J.: Transglutaminase 2 Contributes to Apoptosis Induction in Jurkat T Cells by Modulating Ca²⁺ Homeostasis via Cross-Linking RAP1GDS1. *PLoS One* 8 (12), 14 p., 2013.
DOI: <http://dx.doi.org/10.1371/journal.pone.0081516>
IF:3.534

Total IF of journals (all publications): 13,159

Total IF of journals (publications related to the dissertation): 9,625

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

08 June, 2016



CONFERENCES / ORAL PRESENTATIONS:

Adenosine derived from engulfing macrophages increase the expression of transglutaminase 2 in apoptotic thymocytes via adenosine A2A receptors

8th Molecular Cell and Immune Biology Winter School, Debrecen, Hungary, January 8-10, 2015

Transcriptional control of transglutaminase 2 expression in apoptotic thymocytes

9th Molecular Cell and Immune Biology Winter School, Debrecen, Hungary, January 7-9, 2016

Intracelluláris cAMP szintet növelő vegyületek hatásának vizsgálata a transzglutamináz 2 enzim kifejeződésére egér timusz sejtekben

Debreceni Egyetem Klinikai Orvostudományok Doktori Iskola (KODI) és a Fogorvostudományi Doktori Iskola (FODI) 2014. évi szimpóziuma – Debrecen, 2014. június 16.

CONFERENCES / POSTER PRESENTATIONS

Compounds enhancing intracellular cAMP levels increase the expression of transglutaminase 2 in mouse thymocyte

7nd Molecular Cell and Immune Biology Winter School, Galyatető, Hungary, January 7-10, 2014

Compounds enhancing intracellular cAMP levels increase the expression of transglutaminase 2 in mouse thymocyte

22nd Conference of the European Cell Death Organization, Hersonissos, Crete, Greece, October 1-4, 2014