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

Macrophage polarization signals-induced mechanisms of transcriptional and post-transcriptional repression

by Zsolt Czimmerer

Supervisor: Prof. Dr. Laszlo Nagy

UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF MOLECULAR CELL AND IMMUNE BIOLOGY

DEBRECEN, 2018

Macrophage polarization signals-induced mechanisms of transcriptional and post-transcriptional repression

By Zsolt Czimmerer, MSc

Supervisor: Prof. Dr. Laszlo Nagy, MD, PhD, DSc, member of HAS

Doctoral School of Molecular Cell and Immune Biology, University of Debrecen

Head of the **Examination Committee:** Prof. Dr. Gábor Szabó, MD, PhD, DSc

Members of the Examination Committee: Prof. Dr. Imre Boros, PhD, DSc
Prof. Dr. János Matkó, PhD, DSc

The Examination took place at the Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen
Debrecen; at 11 AM; 23th of May 2017

Head of the **Defense Committee**: Prof. Dr. József Balla, MD, PhD, DSc
Reviewers: Prof. Dr. Eckardt Treuter, PhD
Dr. Péter Bay, PhD, DSc

Members of the Defense Committee: Prof. Dr. Miklós Geiszt, PhD, DSc
Prof. Dr. Péter Nagy, MD, PhD, DSc

The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen
Debrecen; at 13 PM; 19th of April 2018

1. Introduction

1.1. Tissue and molecular microenvironment-dependent heterogeneity of macrophages

Macrophages are important players in the defense against infection and the regeneration following tissue injury and inflammation through the elimination of bacteria, injured host components and apoptotic cells by phagocytosis. They also play complex role in the maintenance of tissue homeostasis and the control of immune response via presentation of antigens, production of cytokines and other biologically active molecules as well as regulation of T-cell activation. Finally, they participate in the resolution of inflammation and wound healing.

The phenotypic and functional properties of macrophages are largely depended on their origin and molecular microenvironment. In adult, the majority of tissue macrophages have embryonic origin deriving from embryonic yolk sac and foetal liver precursors. However, the bone marrow-derived monocytes are also participated in the replenishment of tissue-resident populations with high turnover including gut and are recruited following tissue injury, infection and inflammation. In addition, monocyte recruitment is also observed in the host response to atherogenic, metabolic and nepolastic stimuli participating in the regulation of angigienesis, tumor growth and fibrosis.

The phenotypic and functional plasticity of tissue-resident and monocyte-derived macrophages are depended on their dynamic and partially reversible responsiveness to different macrophage polarizing stimuli such as pathogen-derived molecules as well as cytokines and lipids. The two well characterized extreme outcomes of macrophage polarization are the Th1-type cytokine interferon-gamma (INFγ)-induced classical or M1 macrophage polarization and the Th2-type cytokines interleukin-4 (IL-4)-promoted alternative or M2 macrophage polarization with completely different molecular signatures and functional properties. Nevertheless, recent studies show that the integration of various environmental signals are able to induce an entire spectrum of different polarization states besides the M1/M2 end-points and macrophages can fine-tune their polarization state according to the changing microenvironment during disease progression.

1.2. INFγ and LPS-activated gene expression program during classical macrophages polarization

The classical macrophage polarization is induced by Th1-type cytokine IFNγ and Gram-negative bacterial cell wall component lipopolysaccharide (LPS). IFNγ acts via interferon-gamma receptor 1 (IFNGR1) and IFNGR2 chains-containing IFNγ receptor recruiting Janus kinase 1 (Jak1) and Jak2 adaptors, and activating signal transducer and activator of transcription 1 (STAT1), interferon regulatory factor 1 (IRF-1) as well as IRF-8 transcription factors. IFNγ-activated transcription factors can switch on the classical polarization-specific gene expression program in macrophages promoting the inflammatory response and the elimination of invading pathogens. INFγ-induced C-X-C motif chemokines (CXCLs) such as CXCL9, CXCL10 and

CXCL11 are responsible for the trafficking $CD4^+$ Th1-type T cells, $CD8^+$ T cells and NK cells through their binding to a common receptor C-X-C motif chemokine receptor 3 (CXCR3). INFγ-exposed macrophages produce IL-12 which participates in the differentiation of naive T cells into Th1-type $CD4^+$ T cells [22]. In addition, IL-12 stimulation leads to the enhanced tumor necrosis factor alpha (TNFα) and IFNγ production in T cells and NK cells as well as elevated cytotoxic activity in NK cells and $CD8^+$ cytotoxic T cells. IFNγ-enhanced inducible nitric oxide synthase (iNOS) expression results NO production and elevated anti-bacterial capacities in classically polarized macrophages. INFγ-induced SOCS3 also contributes to the classical macrophage polarization via the attenuation of IL-4 responsiveness and inhibition of alternative macrophage polarization markers.

Gram-negative bacterial cell wall-derived LPS is also an effective inductor of classical macrophage activation. LPS can act as Toll-like receptor 4 (TLR4) ligand and activates nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) and activator protein 1 (AP-1) transcription factor complexes as well as IRFs in myeloid differentiation primary response 88 (MyD88) and/or Toll/IL-1 receptor associated protein (Tirap)-dependent manner. LPS-induced classical macrophage polarization are characterized with the elevated expression of pro-inflammatory cytokines (such as IL-1β, IL-6, IL-12, TNFα and IFNβ), chemokines (CCL2, CXCL10 and CXCL11), antigen presentation molecules as well as co-stimulatory molecules resulting leukocyte trafficking and Th1-type inflammation.

Taken together, the classical macrophage polarization is characterized by production of pro-inflammatory cytokines, reactive nitrogen and oxygen species, induction of Th1 response and elevated microbicidal capacity.

1.3. IL-4 and IL-13-activated gene expression program during alternative macrophages polarization

The alternative macrophage polarization is induced by Th2-type cytokines including IL-4 and IL-13. These cytokines are produced following the activation of Th2-type immune response. The main sources of these cytokines are $CD4^+$ Th2-type T cells, basophil and eosinophil granulocytes as well as macrophages themselves. IL-4 and IL-13 can activate partially different receptor complexes. Both IL-4 and IL-13 bind to IL-4Rα1-γc and IL-4Rα1-IL-13Rα1 heterodimers while IL-13 is also able to bind to IL-13Rα2 chain. The interaction between IL-4 and its receptors lead to the JAK1 and JAK3 activation inducing STAT6 phosphorylation, dimerization and nuclear translocation. The phosphorylated STAT6 dimers initiate the alternative macrophage polarization specific transcriptional program. In addition, further IL-4/STAT6 signaling patway-induced transcriptional factors such as IRF4, Kruppel-like factor4 (KLF4), c-Myc and peroxisome proliferator-activated receptor gamma (PPARγ) also participate in the transcriptional regulation of alternative macrophage polarization. Although, the components of IL-4/STAT6 signaling pathway are same between human and mice, but the IL-4-induced alternatively macrophage polarization markers show species-specific differences. Ym1, Fizz1 and arginase-1 (Arg1) show IL-4-responsiveness in mice, while IL-4-dependent induction of monoamine oxidase A (MAOA) and CD180 expression is restricted to human macrophages. In contrast,

transglutaminase 2 (TGM2), mannose receptor 1 (MRC1/CD206) and PPARγ are common IL-4-induced alternative macrophage polarization markers. Despite the gene expression differences, the IL-4/IL-13-induced alternative macrophage polarization is associated with anti-inflammatory action in both human and mice.

1.4. Macrophage polarization *in vivo*

Although, the gene expression signatures and functional properties of classically and alternatively polarized macrophages are well characterized *in vitro* and *ex vivo*, but the microenvironmental signals-dependent regulation of phenotypic and functional characteristics of macrophages are more complex and less understood *in vivo*. Both classical and alternative macrophage polarization states can be clearly linked to *in vivo* macrophage functions in different physiological and pathological conditions. While the alternative macrophage polarization plays crucial role in the protection against nematode infections and Th2-type inflammation, the classical macrophage activation is tightly associated with Th1-type inflammation and anti-microbial defense. However, several studies showed that M1 and M2 macrophage polarization have overlapping effects in these processes. For instance, classically polarized macrophages-produced reactive nitrogen intermediates may have anti-parasitic activity or alternatively polarized macrophages potentiate the secretion of pro-inflammatory cytokines after *Neisseria meningitides* infection. In addition, inflammatory (M1-like) and restorative (M2-like) macrophages may participate with different kinetics in same pathological process such as muscle injury and regeneration. Nevertheless, the most physiological and pathological processes are characterized *in vivo* by complex immunological milieu. The molecular microenvironment may lead to the development of broad spectrum of mixed macrophage polarization states in different pathological conditions including tumor development and different bacterial infection modulating the disease progression and therapeutic responsiveness. Therefore, the identification of the potential interactions between the different polarization signals is one of the most important challenges in the macrophage biology.

1.5. The epigenomics background of cell type-specific transcriptional regulation

In the last decade, the next generation sequencing-based methods facilitated the better understanding of the connection between the epigenetic code and genomic-regulatory regions including promoters and distal regulatory regions termed enhancers in different cell types. These studies could also give an opportunity for the identification of cell type-specific enhancer repertoire and its responsiveness to different internal or external stimuli.

In general, the enhancers may be located upstream and downstream from the regulated protein-coding or non-coding genes or within their gene bodes and contributes to the transcriptional regulation in collaboration with gene-proximal promoters. The promoters and enhancers are marked by different histone modifications. The promoter regions are generally associated with high level of histone H3 lysine 4 tri-methylation (H3K4m3) as well as low H3K4m1 and H3K4m2. In contrast, the enhancers are

associated with relatively high levels of H3K4m1 and low H3K4m3. The H3K4m1^{high}/H3K4m3^{low} distal regulatory regions are bound by the lineage determining transcription factors (LDTFs), which are responsible for the establishment of cell type-specific enhancer repertoire. These genomic regions serve as an important binding platform for external or internal stimuli-activated stimulus-regulated transcription factors (SRTFs) regulating the cell- and signal-specific transcription in tight collaboration with the LDTFs.
The actual activation state of the enhancers and their transcriptional capacity are strongly depended on the recruited cofactor composition including the ratio of coactivators and corepressors, the histone modification pattern as well as the promoter-enhancer interactions. In addition, recently described that nascent RNA expression is observed at the RNAPII-bound active enhancers in different cell-types. The enhancer RNA (eRNA) expression is dynamically regulated by different external signals, as well as show high correlation with the transcription of neighboring genes suggesting that eRNA expression may be a good marker of enhancer activity. Although, the exact function of eRNAs is not fully understood in the transcriptional regulation, but some findings indicate that eRNAs can participate in the *de novo* promoter-enhancer looping promoting the transcription of protein-coding genes.

1.6. The molecular bases of polarization signals-mediated transcriptional regulation in macrophages

The rapid and dramatic alteration of transcriptional output in macrophages is necessary for the quick adaptation to abruptly changing environments during infections or tissue injuries. In addition, each macrophage subtypes have highly specialized functions in normal homeostatic conditions, which require the expression of function-specific gene subsets. The macrophage-specific transcriptome and its dynamic responsiveness to the microenvironmental changes are significantly determined by different epigeneomic regulatory mechanisms including DNA methylation, post-translational histone modifications and overall 3D architecture of the genome. Based on H3K4m1 and H3K4m3-specific ChIP-seq experiments, 35000-45000 H3K4m1^{high} and H3K4m3^{low} enhancers were identified in mouse macrophages. Importantly, many LDTF binding motifs are enriched significantly at these regions suggesting their important role in the determination of macrophage specific enhancers. Many experimental evidences indicate that PU.1 is a key LDTF for the determination of macrophage-specific enhancer landscape. Moreover, additional LDTFs including CEBPα and IRF8 show highly overlapping cistrome with PU.1 and regulate the development of macrophage-specific enhancer landscape in tight collaboration with PU.1.
The changing molecular microenvironment leads to the activation of SRTFs including NFkB, AP-1, STAT1, STAT6 or nuclear receptors in macrophages. The activated SRTFs control the macrophage polarization-specific gene expression program by binding to the promoters and enhancers of their target genes. In parallel with the SRTF binding, stimulus-induced recruitment of coactivator p300 and enhanced active histone mark H3K27Ac enrichment as well as elevated eRNA expression are also

observed at the enhancers nearby the activated genes indicating their SRTF-dependent activation.
On the other hand, the polarization signals repress large sets of genes, but the repressive activity of polarization-specific transcription factors has not been studied in detail. Importantly, the combination of next generation sequencing-based methods including GRO-seq and ChIP-seq provided opportunities for the characterization of direct transcription factor-mediated repression in different cell types including breast cancer cells and macrophages. It has also been described recently that LPS-dependent activation of macrophages is associated with significantly decreased eRNA expression at several enhancers. These TLR4-signaling-repressed enhancers are characterized by decreased binding capacity of the NF-κB subunit p65, but the exact mechanism of repression remained unknown.

1.7. MicroRNA biogenesis and action

MicroRNAs (miRNAs) are single-stranded, 18-23 nucleotide-long, small non-coding RNA molecules acting as post-transcriptional regulators of gene expression in animals and plants. MiRNAs are located in both intronic/exonic regions of protein-coding genes and intergenic regions. Interestingly, approximately 50% of mammalian miRNAs are organized into miRNA transcription units and generated from primary polycistronic transcripts encoding more than one miRNA. They are usually transcribed by RNAPII. Primary miRNA transcripts (pri-miRNAs) are long (usually over 1 Kb) and incorporate one or more mature miRNA-containing stem-loop structure. These pri-miRNAs undergo several nuclear and cytoplasmic steps of maturation. First, pri-miRNAs are processed in the nucleus by the RNase III-type endonuclease Drosha and its cofactor DGCR8-containing Microprocessor complex. Following the Microprocessor complex-mediated processing, pre-miRNA is exported into the cytoplasm to the completing of miRNA maturation by Exportin 5. Pre-miRNAs are further processed in the cytoplasm by RNase III-type endonuclease Dicer resulting the 20-22 nucleotide-long miRNA/miRNA* duplexes. Finally, the miRNA/miRNA* duplex is loaded onto Argonaute (AGO) proteins forming RNA-induced silencing complex (RISC).
The miRNAs are important components of the post-transcriptional fine-tuning of gene expression in animals and plants. The molecular bases of the interaction between miRNAs and target mRNAs is the complete (in plants) or imperfect (in animals) sequence complementarity. In general, the perfect base paring between the miRNA 5'-proximal seed region (nucleotide 2-8) and mRNA 3'-untranslated region (3'UTR) is required for the stable miRNA:mRNA interaction in metazoan. The miRNAs can cause the inhibition of protein synthesis at both the initiation and post-initiation levels. Besides the translational block, miRNAs are also able to attenuate the RNA stability reducing the "steady-state" mRNA levels of their direct target genes.

1.8. MicroRNAs as the important regulators of macrophage polarization and function

In the last decade, several studies demonstrated that the macrophage-specific miRNome is responsible for different macrophage polarization signals and modulate the phenotypic and functional properties of myeloid cells *in vitro* and *in vivo*. Different inflammatory signals or bacterial pathogens can regulate the miRNA expression signature in macrophages and dendritic cells. These miRNAs participate in the control of inflammatory response and bactericidal capacity of macrophages. The most studied inflammation-induced miRNA is miR-155, which plays a complex role in the macrophages. Mir-155 represses several anti-inflammatory proteins and the alternative macrophage polarization but also inhibits the overwhelmed inflammation via repression of some members of LPS-activated signaling pathways. Another LPS-induced miRNA, miR-147 also represses the different TLR ligands-induced cytokine expression and inflammatory activation of mouse macrophages. Furthermore, LPS-mediated repression of miR-223 directly regulates STAT3 transcription factor expression and promotes IL-6 and IL-1β production in macrophages.
Similarly to inflammatory stimuli, alternative macrophage polarization signals including Th2-type cytokine IL-4 and IL-13 as well as nematode infections are also able to regulate the miRNA expression pattern in macrophages, though their role is less characterized in the control of macrophage phenotype and functions. MiR-125b and miR-378-3p is induced during both nematode infection-induced *in vivo* and IL-4-induced *in vitro* alternative macrophage polarization and the elevated miR-378-3p expression contributes to the proper regulation of *in vivo* proliferation of alternatively polarized macrophages. It has been also shown that IL-4/IL-13-induced miR-193b, miR-124 and miR-511 are participated in the development of alternatively polarized macrophage phenotype. Finally, some recent *in vivo* studies have been shown that the miRNAs play important role in the regulation of functional properties of macrophages in the complex tumor microenvironment.

2. Specific aims

1. Investigation of the IL-4/STAT6 signaling pathway-induced transcriptional repression in mouse macrophages:

- Identifying the gene set repressed by the IL-4/STAT6 signaling pathway.
- Identifying the STAT6-bound enhancer subsets repressed or activated by IL-4.
- Characterizing the direct transcriptional repression mediated by the IL-4/STAT6 signaling pathway.
- Studying the partial repression of inflammatory responsiveness induced by IL-4 pretreatment.

2. Identification of the IL-4 responsive miRNAs and their role in mouse and human alternatively polarized macrophages.

- Investigating the miRNAs in mouse alternatively polarized macrophages.
- Identifying the expression pattern of the selected miRNAs regulated by IL-4 during *in vivo* alternative macrophage polarization in mice.

- Studying the IL-4-dependent regulation of the selected miRNAs in human macrophages.
- Characterizing the molecular bases of IL-4-dependent regulation of the selected miRNAs in mouse and human macrophages.
- Identifying the alternative macrophage polarization-linked function of the selected IL-4 responsive miRNA.

3. Characterization of the transcriptional regulation of inflammation-responsive miRNAs in mouse macrophages.

- Identifying miRNAs regulated at the transcriptional level in LPS-stimulated mouse macrophages.
- Identifying and characterizing the inflammation-responsive pri-miRNAs-associated enhancer subset.
- Investigating the loop formation between the distal-enhancers and promoter of pri-miR-155 regulated by LPS.

3. Materials and Methods

Differentiation of bone marrow-derived macrophages
Bone marrow was isolated from 8-12 weeks old male mice. Isolated bone marrow-derived cells were differentiated for 6 days in the presence of L929 supernatant. For alternative macrophage activation we differentiated the freshly isolated bone-marrow cells in the presence of IL-4 (5 ng/ml) and/or treated BMDMs with IL-4 (20 ng/ml) on the 6^{th} day of the differentiation for the indicated period of time. Differentiated BMDMs were treated with LPS (100 ng/ml) and/or ATP (5mM) for the indicated period of time.

Human monocyte isolation and culture
CD14+ human monocytes were isolated from platelet-free buffy coats from healthy donors by Ficoll gradient centrifugation followed by immunomagnetic cell separation with anti-CD14-conjugated microbeads. Monocytes were cultured in multiwell culture plates in RPMI 1640 supplemented with 10% fetal bovine serum (Invitrogen) and penicillin/streptomycin. For macrophage differentiation and alternative macrophage activation freshly plated monocytes were treated with IL-4 (100 ng/ml). Cells were harvested 12 and 72 hours after cytokine treatment.

RNA-seq
cDNA library for RNA-Seq was generated from 1μg total RNA using TruSeq RNA Sample Preparation Kit according to the manufacturer's protocol. Fragment size distribution and molarity of libraries were checked on Agilent BioAnalyzer DNA1000 chip. Paired read 100bp sequencing runs were performed on Illumina HiScan SQ instrument.

Small RNA-seq
Small RNA-sequencing libraries were generated from 1 μg of total RNA using TruSeq Small RNA Sample Preparation Kit according to manufacturer's protocol. The libraries were checked on BioAnalyzer 2100 using DNA1000 chip. Single read 50bp sequencing was performed on Ilumina HiScan SQ instrument.

ATAC-seq
Cell suspension was diluted to 25k/ml and nuclei were isolated with ATAC-LB. Nuclei from 25k cells were used for tagmentation using Nextera DNA Library Preparation Kit. After tagmentation DNA was purified with Minelute PCR Purification Kit. Tagmented DNA was amplified with Kapa Hifi Hot Start Kit using 9 PCR cycle. Amplified libraries were purified again with Minelute PCR Purification Kit. Fragment distribution of libraries was assessed with Agilent Bioanalyzer and libraries were sequenced on a HiSeq 2500 platform.

GRO-seq
GRO-seq libraries were prepared with NEBNext Small RNA Library Preparation Kit.

ChIP-seq
Libraries were prepared either with Ovation Ultralow Library Systems (Nugen) or TruSeq ChIP library systems (Illumina) according to the manufacturer's instructions. The following antibodies were used: IgG (Millipore, 12-370), H3K27Ac (ab4729), P300 (sc-585), PU.1 (sc-352), JunB (sc-46x), IRF8 (sc-32528x), STAT6 (sc-981), C/EBPα (sc-61X), HDAC1 (ab7028), HDAC2 (ab7029), HDAC3 (ab4729), RNAPII-pS5 (ab5131) and RNAPII-pS2 (ab5095), p65 (sc-372).

3C-seq
After the first digestion and ligation the 3C DNA pool was purified with phenol/chloroform/isoamyl alcohol (25:24:1). Second restriction digestion was performed by using DpnII for 16 hours per manufacturer's instruction. Second ligation was performed at 16C for 6 hours with 200U of T4 DNA ligase. DNA was then purified again with phenol/chloroform/isoamyl alcohol (25:24:1) followed by QIAquick gel purification columns (Qiagen). Bait specific inverse PCRs were performed using primers coupled to Universal Illumina adapters and Barcode sequences. Amplicon libraries were quantified and qualified by Agilent using DNA 7500 chip cartridge. Amplicon libraries were sequenced on Illumina HiSeq sequencer.

LDH release
LDH activity was measured in the supernatants of unstimulated and IL-4-pretreated WT and STAT6KO bone marrow-derived macrophages after IL-4 pretreatment and/or LPS/ATP costimulation (LPS-exposed BMDMs were treated with ATP for 30 min) by commercially available LDH UV assay on Cobas c 501 instrument.

Measurement of IL-1β production

IL-1β was measured from samples using ELISA kit according to the manufacturer's instructions.

Western Blot analysis
Proteins were separated by SDS-PAGE and transferred onto nitrocellulose membranes. The following antibodies were used: Pro-IL-1β (AF401-NA), ASC (sc22514-R), pro-caspase-1 (AG-20B-0042), NLRP3 (AG-20B-0014) and β-actin.

Caspase-1 assay and PI-based detection of pyroptotic cell death
Caspase-1 activity and pyroptotic cell death by propidium iodine staining was measured in single cells using imaging Laser Scanning Cytometry (LSC).

Cell number analysis
Macrophages attached to the bottom of 96-well plates were permeabilized by Triton X-100, stained by PI (propidium iodide) and measured in a Synergy HT microplate reader. Cell numbers were determined using cell number reference curve.

Cell viability analysis
Resazurin-based viability staining (Promega) was carried out according to manufacturer's protocol.

Cell cycle analysis
Raw264.7 cells were fixed on chamber slides using methanol. Fixed cells were stained with propidium iodide. Cell cycle distribution was measured in single cells using imaging Laser Scanning Cytometry (LSC).

Apoptosis assay
48 hours following miRNA transfection, the cells were labeled by Hoechst 33342, propidium iodide and FITC-conjugated Annexin V. Apoptotic cell death was measured in single cells using imaging Laser Scanning Cytometry (LSC).

4. Results

4.1. IL-4-STAT6 signaling pathway-mediated direct transcriptional repression limits inflammatory responsiveness in alternatively polarized macrophages

4.1.1. IL-4/STAT6 signaling pathway represses a large set of genes at the transcriptional level

In this study, we used the combination of different next generation sequencing-based methods for the characterization of IL-4/STAT6-signaling mediated transcriptional regulation in mouse macrophages. We identified four IL-4-induced gene expression clusters with different fold changes and expression kinetics. Moreover, we observed that a relatively high portion of IL-4-responsive genes were repressed. Importantly, IL-4-dependent repression and activation were completely abolished in the STAT6-

deficient macrophages. RNAPII binding, H3K27Ac enrichment and nascent RNA expression were regulated similarly to the steady-state mRNA level at the gene bodies of IL-4-activated and repressed gene clusters following short IL-4 exposure. Taken together, these results indicate that IL-4/STAT6 signaling pathway directly activates and represses gene expression, primarily at the transcriptional level during the alternative macrophage polarization.

4.1.2. IL-4-activated STAT6 binding is required for the direct transcriptional activation and repression

IL-4-activated STAT6 were bound to 20119 genomic regions in IL-4-exposed mouse macrophages. Based on IL-4-regulated RNAPII-pS5 binding, the RNAPII-pS5 positive STAT6 peaks were divided into three sub-groups: "repressor", "neutral" and "activator" STAT6 peak clusters. IL-4-dependent regulation of RNAPII-pS2 binding and H3K27Ac enrichment exhibited similar patterns to RNAPII-pS5 in all three STAT6 clusters. Finally, the "repressor" STAT6 peaks were tightly associated with the IL-4-repressed gene cluster, while "activator" STAT6 peaks were linked to the immediate early IL-4-induced genes containing gene expression clusters. Collectively, these results suggest a tight connection between STAT6-dependent regulation of enhancer activity and neighboring gene expression in the same genomic loci.

4.1.3. STAT6 binding has different characteristics at the repressed and activated enhancers

The majority of STAT6-bound genomic sites were detected in intergenic and intronic regions in the genome in all three clusters. The active enhancer mark H3K4m1 enrichment was detected on more than 98% of STAT6-bound genomic sites, but it was not changes significantly following IL-4 treatment. These findings suggest that STAT6 transcription factor primarily binds to enhancers. Interestingly, the “repressor” and "neutral" STAT6 peaks were associated with typically lower occupancies compared to the IL-4-induced RNAPII-pS5-associated "activator" STAT6 peaks. In addition, both the under-representation of canonical STAT6 motif and lower STAT6 motif score were observed under "repressor" and "neutral" STAT6 peaks compared to the "activator" STAT6 peaks. Considering that the presence of STAT6 is needed for repression, these findings suggest that STAT6 is bound without direct DNA contact or it recognizes non-canonical binding motifs at the repressed genomic regions.

4.1.4. STAT6-mediated repression of enhancer activity is associated with decreased chromatin accessibility and linage-determining transcription factor binding

Chromatin accessibility determines enhancer activity in different cell types. Furthermore, binding of macrophage LDTFs such as PU.1, JUNB, C/EBPα and IRF8

are associated with active enhancers in macrophages. Elevated chromatin accessibility was observed at the “activator” STAT6-bound sites, while its significant reduction was detected at the “repressor” STAT6-bound genomic regions following short-term IL-4-exposure. High portion of the STAT6 peaks were overlapped with the examined LDTF cistromes in all three STAT6 peak clusters except for JUNB which showed moderated overlap. Interestingly, PU.1, JUNB and C/EBPα binding were significantly diminished, while IRF8 binding was not changed at the "repressor" STAT6-bound genomic regions after 1 hour IL-4 treatment in mouse macrophages. In contrast, all four LDTFs showed significantly increased binding at the activated STAT6-bound enhancers following IL-4 stimulation. These findings suggest that both STAT6-mediated enhancer activation and repression are associated with the alteration of chromatin openness and LDTFs binding in the alternatively polarized macrophages.

4.1.5. IL-4/STAT6 signaling pathway-mediated repression of enhancers is characterized by an altered p300:HDAC ratio

The acetylation status and thus the activity of enhancers are tightly regulated by histone acetyl-transferases such as p300 and classical histone deacetylase (HDAC) enzymes including HDAC1, 2 and 3. The majority of STAT6-bound genomic sites were either pre-loaded by p300 and classical HDACs or recruited these factors upon IL-4 stimulation. The binding of p300 was significantly induced at the "activator" STAT6-bound genomic regions, but significantly decreased at STAT6-repressed enhancers after 1 hour of IL-4 treatment. Unexpectedly, classical HDACs occupancy were significantly increased at STAT6-activated enhancers in IL-4-stimulated BMDMs. In contrast, STAT6-repressed enhancers were bound by HDAC1, 2 and 3 at the basal state and their occupancy was not changed significantly by IL-4. Taken together, these findings show that STAT6-repressed enhancers are bound by both p300 and classical HDACs at the steady-state, and p300 binding is selectively decreased by IL-4 resulting in a modified equilibrium favoring HDAC binding.

4.1.6. The presence of HDAC3 is required for IL-4/STAT6-signaling pathway-mediated repression on a subset of genes

Direct interactions between STAT transcription factors and classical HDACs have been described previously in numerous cell types regulating STAT transcription factors-mediated direct transcriptional regulation. Furthermore, HDAC3 has been shown to contribute to the control of alternative macrophage polarization *in vitro* and *in vivo*. We found that a specific subset of IL-4-repressed genes (371/1628) showed diminished repression in HDAC3-deficient macrophages following IL-4 exposure. Interestingly, 325 STAT6-repressed enhancers were observed in the same sub-TAD with IL-4/HDAC3-repressed genes. These genomic regions were bound by HDAC3, but HDAC3 occupancy was not changed by IL-4 treatment. It has been described previously that HDAC3 is one of the key components of NCoR/SMRT corepressor complex. Indeed, the IL-4/STAT6/HDAC3 repressed enhancer set was also bound by both NCoR and SMRT in unstimulated BMDMs. Finally, our analyses demonstrated that the IL-4/STAT6/HDAC3-mediated repression of four selected genes including

Fos, Lyz1, Lyz2 and Smad3 was partially NCoR dependent. Taken together, our results indicate that IL-4/STAT6 signaling pathway induces transcriptional repression via NCoR/HDAC3 complex at a subset of genes. Thus, we explored at least one of the molecular mechanisms for STAT6-dependent transcriptional repression.

4.1.7. IL-4/STAT6-mediated direct transcriptional repression affects the LPS-induced inflammatory program of macrophages

The significant overrepresentation of the inflammation-linked signaling pathways including NOD-like receptor signaling and Toll-like receptor signaling pathways was restricted to IL-4-repressed genes. In addition, the majority of IL-4-repressed genes including several members of NOD-like and Toll-like receptor signaling pathways indicated reduced mRNA expression following 24 hours of IL-4 exposure accompanied by decreased STAT6 binding at the repressed enhancers. These results raised the possibility that IL-4 can influence the subsequent inflammatory response of the macrophages via directly repressed enhancers following the dissociation of STAT6.
For the characterization of IL-4/STAT6-modulated inflammatory program, IL-4-pre-treated (24h) and LPS-activated mouse macrophages were used. Interestingly, 520 out of 1350 LPS-activated genes including several members of the NOD-like receptor and Toll-like receptor signaling pathways showed IL-4 pre-treatment-attenuated inflammatory responsiveness. RNAPII-pS2 binding showed a similar pattern to "steady-state" mRNA level suggesting that IL-4 pre-treatment influences LPS-activated gene expression primarily at the transcriptional level. Finally, the RT-qPCR-based measurement of Tlr2, Nlrp3, Cd14 and Clec4d expression indicated that IL-4-dampended inflammatory responsiveness was completely STAT6 dependent. These results raise the possibility that IL-4-activated STAT6 can directly influence the inflammatory responsiveness in macrophages via transcriptional repression of certain components of the LPS-activated inflammation-specific gene expression program.

4.1.8. IL-4/STAT6 signaling pathway-dependent attenuation of inflammatory responsiveness on a specific subset of enhancers

961 enhancers were found with overlapping IL-4-activated STAT6 and LPS-activated p65 peaks in the sub-TADs of IL-4-repressed LPS inducible genes. 641 out of 961 genomic regions showed significantly increased RNAPII-binding after LPS activation. Interestingly, 70% (448/641) of LPS-activated enhancers were associated with significantly attenuated basal and LPS-induced RNAPII binding following 24 hours of IL-4 pre-treatment. Based on p65 binding, these enhancers were divided into two different subsets including "IL-4-insensitive" and "IL-4-attenuated" p65 binding-associated enhancers. Finally, both the IL-4-attenuated enhancer activity and p65 binding proved to be completely STAT6 dependent at the selected enhancers. Taken together, these results indicate that the activation of IL-4/STAT6 signaling pathway can reduce the inflammatory responsiveness of macrophages through selective, direct repression of a distinct LPS-inducible enhancer set.

4.1.9. IL-4-mediated repression of inflammatory response results in attenuated inflammasome activation, decreased IL-1β production and pyroptosis

Genes showing opposing regulation by IL-4 and LPS were mostly associated with inflammation-linked pathways, including NOD-like and Toll-like receptor signaling pathways. It is known that NOD-like receptors play central role in the inflammasome activation leading to IL-1β secretion and inflammasome-associated cell death, pyroptosis. Similarly to Nlrp3, the basal and LPS-induced Il-1b expression was repressed directly at transcriptional level by IL-4/STAT6 signaling pathway. In addition, LPS-induced NLRP3 and pro-IL-1β expression were also reduced at the protein level by IL-4/STAT6 signaling pathway, while the expression of additional inflammasome components such as proCaspase-1 and ASC was not changed after IL-4 and LPS stimulation of mouse macrophages. As expected, LPS/ATP stimulation-induced Caspase-1 activity, IL-1β secretion and pyroptotic cell death were attenuated in IL-4-pre-treated macrophages in a STAT6 dependent manner. Finally, both Nlrp3 and Il1b expression showed similar regulation in the *in vivo* model of the alternative macrophage polarization-followed inflammation. Taken together, these findings suggest that prior *in vitro* or *in vivo* alternative macrophage polarization can modulate the subsequent inflammatory response of macrophages, including inflammasome activation, IL-1β secretion as well as pyroptosis through the direct repression of *Nlrp3* and *Il1b* gene expression by IL-4/STAT6 signaling pathway.

4.2. The IL-4/STAT6 signaling pathway orchestrates a conserved microRNA signature in human and mouse alternatively polarized macrophages regulating cell survival via miR-342-3p

4.2.1. Identification of the IL-4/STAT6 signaling pathway responsive miRNA signature during mouse alternatively macrophage polarization

Applying small RNA-seq method, 162 IL-4-repsonsive miRNAs (76 upregulated and 81 downregulated) were identified in alternatively polarized wild-type macrophages compared to non-polarized controls. Although, the majority of the IL-4-responsible miRNAs (151 from 157) were absolutely STAT6-dependent, but some miRNAs showed significantly regulated expression level following IL-4 exposure in STAT6-deficient macrophages. Interestingly, the three selected IL-4/STAT6 signaling pathway responsive miRNAs including the activated miR-342-3p and repressed miR-125a and 99b were similarly regulated in the *Brugia malayi* implantation-induced *in vivo* alternative macrophage polarization model. Collectively, these results indicate that macrophage miRNome are regulated intensively via the IL-4/STAT6 signaling pathway during *in vitro* and *in vivo* alternative macrophage polarization.

4.2.2. Direct STAT6-dependent co-regulation of miR-342-3p and EVL expression in mouse and human macrophages

MiR-342-3p is located within the third intron of the EVL gene in humans and mice, and their expression showed coordinated regulation in different tumor types. Both miR-342-3p and its host gene were induced in human and mouse macrophages by IL-4. In addition, STAT6 binding were also detected in the EVL/Evl loci in IL-4-exposed human and mouse macrophages. These results suggest that miR-342-3p and its host gene EVL are directly co-regulated in both mouse and human macrophages by IL-4/STAT6 signaling pathway.

4.2.3. MiR-342-3p acts as a regulator of macrophage cell number via reduction of cell viability and induction of apoptosis

The proliferation of local macrophage population is induced *in vivo* by IL-4-mediated Th2-type inflammation. MiR-342-3p is aberrantly downregulated in different tumor types including colorectal and breast cancers as well as its overexpression is able to induce apoptosis and block cancer cell proliferation. The combination of our transcriptomics, computational and functional approaches showed that miR-342-3p overexpression could induce the apoptotic cell death of RAW264.7 macrophages via the repression of anti-apoptotic gene network attenuating viable macrophage number. In addition, the direct interaction between miR-342-3p and anti-apoptotic Bcl2l1 was also demonstrated. Collectively, these findings raise the possibility that miR-342-3p acts as a negative feedback regulator of IL-4-induced local macrophage proliferation.

4.3. Dynamic transcriptional control of macrophage miRNA signature via inflammation responsive enhancers revealed using a combination of next generation sequencing-based approaches

4.3.1. Characterization of the transcriptional basis of inflammation responsive miRNA signature in macrophages

17 significantly regulated mature miRNAs (4 downregulated and 13 upregulated) were found in mouse macrophages following 3h LPS exposure. 15 LPS-responsive miRNAs including miR-155, miR-147 and miR-223 showed similar expression patterns with their pri-miRNAs suggesting that the majority of inflammation responsive miRNAs are regulated at the transcriptional level.

4.3.2. Transcription start sites of inflammation responsive pri-miRNAs are associated with general active promoter marks and RNA polymerase II binding

It is well characterized that specific epigenetic marks including H3K27Ac and H3K4m3 as well RNAPII binding are associated with the promoters of transcriptionally active protein-coding genes. The active promoter-specific H3K4m3 enrichment and RNAPII binding have been also used for the identification of pri-

miRNA promoter regions. As expected, the TSSs of the inflammation-regulated pri-miRNA genes were associated with H3K4m3, H3K27Ac and RNAPII enrichments. LPS stimulation induced H3K27Ac enrichment and RNAPII binding on the TSSs of LPS-activated pri-miRNAs. In contrast, LPS-dependent attenuation of H3K27Ac enrichment and RNAPII binding were observed at the TSSs of inflammation-repressed pri-miRNA, without the alteration of H3K4m3 enrichment. Taken together, these findings suggest that (i) LPS sensitive miRNA genes have similar epigenetic features as protein-coding genes and (ii) their transcription seems to be dependent on RNAPII.

4.3.3. LPS-regulated pri-miRNAs are associated with an inflammation-responsive enhancer network

The protein-coding genes-associated enhancers are marked by specific post-translational histone modifications including H3K4m1, H3K4m2 and eRNA expression. Enhancers have also been described to regulate pri-miRNA expression in many cell types and tissues. These pri-miRNA-linked distal regulatory regions have general enhancer-like features including enhancer-specific H3K4m1 enrichment and eRNA expression. As expected, the LPS responsive pri-miRNA genes were associated with H3K4m1 and eRNA "positive" enhancers. Our global analysis identified 33 induced and 11 repressed distal regulatory regions in the sub-TADs of LPS-regulated pri-miRNA genes indicating that LPS-sensitive miRNA genes are associated with distal regulatory regions showing similar LPS-mediated eRNA expression kinetics with pri-miRNA expression. These observations are consistent with studies focusing on mRNA-associated enhancers, thus indicate that enhancers are able to participate in the transcriptional regulation of the inflammation responsive miRNA expression, analogous to mRNAs.

4.3.4. The genome architectural context of inflammation-induced pri-miR-155 expression

Mapping of the pri-miR-155-associated, LPS-responsive enhancers showed that these enhancers are located far away (between -54 and -116 Kb) from the TSS of pri-miR-155-coding genomic region. Using 3C-seq experiments, both constitutive and LPS-induced interactions were identified within the sub-TAD of pri-miR-155. Permanent interaction was detected between the TSS of pri-miR-155 and the -116Kb enhancer. Furthermore, LPS-triggered interactions were observed between pri-miR-155_-116 Kb and other LPS-activated enhancers within the sub-TAD. Collectively, these results indicated that (i) the identified inflammation activated enhancers interact with each other and the TSS of pri-miR-155; (ii) the architecture of pri-miR-155-linked sub-TAD undergoes inflammation-mediated spatial reorganization in mouse macrophages.

5. Discussion

The functional diversity and plasticity of macrophages are largely depended on the microenvironmental signals including inflammatory mediators, cytokines and lipids both in physiological and pathological conditions. In the last decade, many microenvironmental signals-induced molecular and epigenetic mechanisms including SRTF-mediated transcriptional activation or miRNA-mediated post-transcriptional repression have been intensively characterized in macrophages. However, many issues remain unanswered in connection with macrophage polarization signals-activated transcriptional and post-transcriptional regulatory mechanisms. On the one hand, large gene sets are repressed by macrophage polarization signals, but the mechanisms of SRTF activation-dependent repression is largely unknown. On the other hand, although the biological functions of macrophage polarization signals-responsive miRNAs are well studied, their transcriptional regulation remains poorly understood.

Our systematic genome-wide analysis revealed that the alternative macrophage polarization signal IL-4 represses a large gene set at the transcriptional level. IL-4-activated STAT6 binds the repressed genes-linked distal regulatory elements. The STAT6-binding was associated with reduced chromatin openness, LDTF, p300 and RNAPII binding as well as decreased eRNA expression. These results suggest that the IL-4-activated STAT6 transcriptional factor can act as a transcriptional repressor in macrophages. In addition, we found that the IL-4/STAT6 signaling pathway-mediated transcriptional repression diminishes the inflammatory responsiveness of macrophages including NLRP3 inflammasome activation, IL-1β production and pyroptosis. Taken together, these results show that complex bidirectional interactions exist between alternative macrophage polarization and inflammatory signals which influence the responsiveness and sensitivity of macrophages toward microbial-, stress-, and damage-associated endogenous signals.

Here, we also observed the direct transcriptional regulation of miR-342-3p and their host gene EVL in human and macrophages by IL-4/STAT6 signaling pathway. We demonstrated that miR-342-3p overexpression can reduce macrophage survival via the induction of apoptotic cell death. In addition, we identified a miR-342-3p-targeted anti-apoptotic gene network using a combination of transcriptomics, computational and functional approaches. These results indicate that IL-4/STAT6 signaling pathway-induced miR-342-3p potentially participates in the negative feed-back regulation of IL-4-mediated macrophage proliferation.

Finally, we studied the LPS-dependent transcriptional regulation of macrophage miRNome including miR-155, miR-147 and miR-223 using an integrated NGS-based approach. Here we showed that LPS-dependent transcriptional induction of miR-155 expression is based on an intensive communication between the distal enhancers and the pri-miR-155-linked TSS and associated with the reorganization of the pri-miR-155-coding genomic locus.

6. Summary

The complex molecular microenvironment tightly determines the phenotypic and functional features of macrophages in different physiological and pathological conditions. The macrophage exposure to different polarization signals including cytokines and pathogen-derived molecules leads to the activation of signal specific gene expression program via SRTFs. In our work, we showed that IL-4/STAT6 signaling pathway can repress a large gene set at the transcription level during mouse alternative macrophage activation. We demonstrated that this mechanism is able to modulate the inflammatory responsiveness of IL-4-exposed macrophages attenuating of LPS-induced inflammasome activation, IL-1β production and pyroptosis.

The miRNA-mediated post-transcriptional repression is also contributed to the regulation of macrophage polarization and function. Here, we identified that the IL-4/STAT6 signaling pathway-induced miR-342-3p reduces a viable macrophage number via the repression of anti-apoptotic gene pathway potentially participating in the negative feed-back regulation of IL-4-mediated macrophage proliferation.

Finally, we characterized the transcriptional regulation of the inflammation responsive miRNome in mouse macrophages with the combination of NGS-based genomic approaches showing the important role of inflammatory signals-regulated enhancers in the control of miRNA expression and providing a useful tool for the study of transcriptional regulation of miRNA expression in other cellular systems.

7. Publications Related to Dissertation

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRAY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/28/2018.PL
Subject: PhD Publikációs Lista

Candidate: Zsolt Czimmerer
Neptun ID: J06DZW
Doctoral School: Doctoral School of Molecular Cellular and Immune Biology
MTMT ID: 10038295

List of publications related to the dissertation

1. Czimmerer, Z., Horváth, A., Dániel, B., Nagy, G., Cuaranta-Monroy, I., Kiss, M., Kolostyák, Z., Póliska, S., Steiner, L., Giannakis, N., Varga, T., Nagy, L.: Dynamic transcriptional control of macrophage miRNA signature via inflammation responsive enhancers revealed using a combination of next generation sequencing-based approaches.
Biochim. Biophys. Acta-Gene Regul. Mech. 1861 (1), 14-28, 2018.
DOI: http://dx.doi.org/10.1016/j.bbagrm.2017.11.003
IF: 5.018 (2016)

2. Czimmerer, Z., Dániel, B., Horváth, A., Rückerl, D., Nagy, G., Kiss, M., Peloquin, M., Budai, M. M., Cuaranta-Monroy, I., Simándi, Z., Steiner, L., Nagy, B., Póliska, S., Bankó, C., Bacsó, Z., Schulman, I. G., Sauer, S., Deleuze, J. F., Allen, J. E., Benkő, S., Nagy, L.: The Transcription Factor STAT6 Mediates Direct Repression of Inflammatory Enhancers and Limits Activation of Alternatively Polarized Macrophages.
Immunity. 48 (1), 75-90, 2018.
DOI: http://dx.doi.org/10.1016/j.immuni.2017.12.010
IF: 22.845 (2016)

3. Czimmerer, Z., Varga, T., Kiss, M., Vázquez, C. O., Doan-Xuan, Q. M., Rückerl, D., Tattikota, S. G., Yan, X., Nagy, Z. S., Dániel, B., Póliska, S., Horváth, A., Nagy, G., Varallyay, É., Poy, M. N., Allen, J. E., Bacsó, Z., Abreu-Goodger, C., Nagy, L.: The IL-4/STAT6 signaling axis establishes a conserved microRNA signature in human and mouse macrophages regulating cell survival via miR-342-3p.
Genome Med. 8 (1), 1-22, 2016.
DOI: http://dx.doi.org/10.1186/s13073-016-0315-y
IF: 7.071

8. List of Other Pubications

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRAY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

List of other publications

4. Czimmerer, Z., Nagy, Z. S., Nagy, G., Horváth, A., Silye-Cseh, T., Kriston, Á., Jonás, D., Sauer, S., Steiner, L., Dániel, B., Deleuze, J. F., Nagy, L.: Extensive and functional overlap of the STAT6 and RXR cistromes in the active enhancer repertoire of human CD14+ monocyte derived differentiating macrophages.
Mol. Cell. Endocrinol. [Epub ahead of print], 2017.
DOI: http://dx.doi.org/10.1016/j.mce.2017.07.034
IF: 3.754 (2016)

5. Fejes, Z., Póliska, S., Czimmerer, Z., Káplár, M., Penyige, A., Gál Szabó, G., Beléné Debreceni, I., Kunapuli, S. P., Kappelmayer, J., Nagy, B.: Hyperglycemia suppresses microRNA expression in platelets to increase P2RY12 and SELP levels in type 2 diabetes mellitus.
Thromb. Haemost. 117 (3), 529-542, 2017.
DOI: http://dx.doi.org/10.1160/TH16-04-0322
IF: 5.627 (2016)

6. Kiss, M., Czimmerer, Z., Nagy, G., Bieniasz-Krzywiec, P., Ehling, M., Pap, A., Póliska, S., Botó, P., Tzerpos, P., Horváth, A., Kolostyák, Z., Dániel, B., Szatmári, I., Mazzone, M., Nagy, L.: Retinoid X receptor suppresses a metastasis-promoting transcriptional program in myeloid cells via a ligand-insensitive mechanism.
Proc. Natl. Acad. Sci. U. S. A. 114 (40), 10725-10730, 2017.
DOI: http://dx.doi.org/10.1073/pnas.1700785114
IF: 9.661 (2016)

7. Gyöngyösi, A., Dócs, O., Czimmerer, Z., Orosz, L., Horváth, A., Török, O., Méhes, G., Nagy, L., Bálint, B. L.: Measuring expression levels of small regulatory RNA molecules from body fluids and formalin-fixed, paraffin-embedded samples.
Methods Mol. Biol. 1182, 105-119, 2014.
DOI: http://dx.doi.org/10.1007/978-1-4939-1062-5_10

8. Dániel, B., Nagy, G., Hah, N., Horváth, A., Czimmerer, Z., Póliska, S., Gyuris, T., Keirsse, J., Gysemans, C., Van Ginderachter, J. A., Bálint, B. L., Evans, R. M., Barta, E., Nagy, L.: The active enhancer network operated by liganded RXR supports angiogenic activity in macrophages.
Genes Dev. 28 (14), 1562-1577, 2014.
DOI: http://dx.doi.org/10.1101/gad.242685.114
IF: 10.798

9. Czimmerer, Z., Hulvely, J., Simándi, Z., Varallyay, É., Havelda, Z., Szabó, E., Varga, A., Dezső, B., Balogh, M., Horváth, A., Domokos, B., Török, Z., Nagy, L., Bálint, B. L.: A versatile method to design stem-loop primer-based quantitative PCR assays for detecting small regulatory RNA molecules.
PLoS One. 8 (1), 1-10, 2013.
DOI: http://dx.doi.org/10.1371/journal.pone.0055168
IF: 3.534

10. Nagy, Z., Czimmerer, Z., Nagy, L.: Nuclear receptor mediated mechanisms of macrophage cholesterol metabolism.
Mol. Cell. Endocrinol. 368 (1-2), 85-98, 2013.
DOI: http://dx.doi.org/10.1016/j.mce.2012.04.003
IF: 4.241

11. Nagy, Z., Czimmerer, Z., Szántó, A., Nagy, L.: Pro-inflammatory cytokines negatively regulate PPARγ mediated gene expression in both human and murine macrophages via multiple mechanisms.
Immunobiology. 218 (11), 1336-1344, 2013.
DOI: http://dx.doi.org/10.1016/j.imbio.2013.06.011
IF: 3.18

12. Brignull, L. M., Czimmerer, Z., Saidi, H., Dániel, B., Villela, I., Bartlett, N. W., Johnston, S. L., Meira, L. B., Nagy, L., Nohturfft, A.: Reprogramming of lysosomal gene expression by interleukin-4 and Stat6.
BMC Genomics. 14 (1), 1-20, 2013.
DOI: http://dx.doi.org/10.1186/1471-2164-14-853
IF: 4.041

13. Kiss, M., Czimmerer, Z., Nagy, L.: The role of lipid-activated nuclear receptors in shaping macrophage and dendritic cell function: from physiology to pathology.
J. Allergy Clin. Immunol. 132 (2), 264-286, 2013.
DOI: http://dx.doi.org/10.1016/j.jaci.2013.05.044
IF: 11.248

14. Czimmerer, Z., Varga, T., Póliska, S., Német, I., Szántó, A., Nagy, L.: Identification of novel markers of alternative activation and potential endogenous PPARgamma ligand production mechanisms in human IL-4 stimulated differentiating macrophages.
Immunobiology. 217 (12), 1301-1314, 2012.
DOI: http://dx.doi.org/10.1016/j.imbio.2012.08.270
IF: 2.814

15. Varga, T., Czimmerer, Z., Nagy, L.: PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation.
Biochim. Biophys. Acta Mol. Basis Dis. 1812 (8), 1007-1022, 2011.
DOI: http://dx.doi.org/10.1016/j.bbadis.2011.02.014
IF: 5.387

16. Oberoi, J., Fairall, L., Watson, P. J., Yang, J. C., Czimmerer, Z., Kampmann, T., Goult, B. T., Greenwood, J. A., Gooch, J. T., Kallenberger, B. C., Nagy, L., Neuhaus, D., Schwabe, J. W. R.: Structural basis for the assembly of the SMRT/NCoR core transcriptional repression machinery.
Nat. Struct. Mol. Biol. 18 (2), 177-184, 2011.
DOI: http://dx.doi.org/10.1038/nsmb.1983
IF: 12.712

17. Mikó, E., Margitai, Z., Czimmerer, Z., Várkonyi, I., Dezső, B., Lányi, Á., Bacsó, Z., Scholtz, B.: miR-126 inhibits proliferation of small cell lung cancer cells by targeting SLC7A5.
FEBS Lett. 585 (8), 1191-1196, 2011.
DOI: http://dx.doi.org/10.1016/j.febslet.2011.03.039
IF: 3.538

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRAY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

18. Mikó, E., Czimmerer, Z., Csánky, E., Boros, G., Buslig, J., Dezső, B., Scholtz, B.: Differentially expressed microRNAs in small cell lung cancer.
Exp. Lung Res. 35 (8), 646-664, 2009.
DOI: http://dx.doi.org/10.3109/01902140902822312
IF: 1.177

19. Rőszer, T., Jenei, Z., Gáll, T., Nagy, O., Czimmerer, Z., Serfőző, Z., Elekes, K., Bánfalvi, G.: A possible stimulatory effect of FMRFamide on neural nitric oxide production in the central nervous system of Helix lucorum L.
Brain. Behav. Evol. 63 (1), 23-33, 2004.
IF: 1.954

20. Rőszer, T., Czimmerer, Z., Szentmiklósi, J. A., Bánfalvi, G.: Nitric oxide synthesis is blocked in the enteral nervous system during dormant periods of the snail Helix lucorum L.
Cell Tissue Res. 316, 255-262, 2004.
IF: 2.67

21. Rőszer, T., Jenei, Z., Serfőző, Z., Czimmerer, Z., Bánfalvi, G.: Structural diversity of NADPH diaphorase-reactive enteral networks in Stylommatophora (Gastropoda, Pulmonata).
Invertebr. Biol. 123 (2), 128-135, 2004.
IF: 0.754

Total IF of journals (all publications): 122,024
Total IF of journals (publications related to the dissertation): 34,934

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.

26 January, 2018