Investigation of Angiotensin II-induced Gene Expression Changes In Vascular Smooth Muscle Cells

Ph.D. thesis

Janka Borbála Gém, MD

Semmelweis University Doctoral School
Molecular Sciences Division

Supervisors: László Hunyady, MD, Ph.D, D.Sc
András Balla, Ph.D, D.Sc

Official reviewers: Fagyas Miklós, MD, Ph.D
Dézsi László MD, Ph.D, Med. Habil.

Head of the Complex Examination Committee:
Szabolcs Várbíró MD, Ph.D, Med. Habil.

Members of the Complex Examination Committee:
Éva Ruisanchez, MD, Ph.D
Anita Alexa, Ph.D

Budapest, 2026

1. Introduction

Angiotensin II (AngII) is a peptid type hormone, responsible for the salt-water homeostasis of the human body and the regulation of systemic blood pressure. In vascular smooth muscle cells (VSMCs), binding to angiotensin type 1 receptor (AT1R), AngII initiates various short- and long-term physiological responses. In short-term, AngII causes quick vasoconstriction that contributes to the elevation of the systemic blood pressure. Its long-term effects are mainly related to gene expression changes, resulting cellular proliferation, hypertrophy, cellular migration and fibrosis, which processes can highly contribute to pathophysiological changes in the vasculature, such as vascular remodeling, hypertension or atherosclerosis.

During my Ph.D research, I investigated the gene expression changes, provoked by AngII in primary rat VSMCs. Numerous genes were identified, responding with up- or downregulation for AngII stimuli. We found LIM and cysteine rich domains 1 (LMCD1), a transcriptional cofactor, known to play a role in different proliferative processes, to be one of the most upregulated genes. As there is a limited amount of literature data about LMCD1, we aimed to investigate the pathways leading to its upregulation by AngII and also its physiological roles in vascular smooth muscle cells.

Additionally, we performed pathway analysis to determine the most important intracellular signaling pathways, leading to AngII mediated gene expression changes in primary rat VSMCs. Due to our results, mitogen activated protein kinase (MAPK) and epithelial growth factor receptor (EGFR) pathways showed the highest activity. It is widely known that EGFR transactivation of AT1R plays a significant role in forming the long-term effects of AngII. Earlier studies examined EGFR transactivation mainly using pharmacological inhibition of the receptor. It has to be mentioned that

pharmacological inhibitors often inhibit off-target molecules which can provide misleading results. To eliminate these potential errors, we created a lentiviral system that silences the transcription of *EGFR* gene which method was aimed to provide us more specific inhibition. Among the genes, upregulated by AngII in VSMCs, we identified three different dual specificity phosphatase (DUSP) isoforms, each from different subtype from DUSP proteins, nominatedly *DUSP5*, *DUSP6* and *DUSP10*. DUSPs are the most important regulators of the MAPK activity, so they can potentially have a role in forming the long-term effects of AngII. During our research, we investigated the importance of EGFR transactivation through the AngII mediated upregulation of the three mentioned DUSP isoforms, comparing the effects of the pharmacological inhibition and genetical silencing of EGFR pathway.

2. Objectives

The aim of this PhD research was to investigate genes that exhibit upregulation in response to AngII stimulation in primary rat vascular smooth muscle cells, with a particular focus on the role of EGFR transactivation mediated by the AT1R. The objectives are organized into two major areas:

2.1. Aims related to AngII-mediated gene expression changes:

- To identify genes that are significantly upregulated by AngII in primary rat VSMCs.
- To elucidate the signaling mechanisms leading to the AngII-mediated upregulation of *LMCD1*
- Characterization of the physiological effects of LMCD1 in VSMCs.

2.2. Aims related to EGFR transactivation:

- Silencing *EGFR* with a lentiviral vector system effectively in primary rat VSMCs.
- To examine the effects of *EGFR* silencing on AngII-induced upregulation of *DUSP5*, *DUSP6* and *DUSP10* compared to pharmacological EGFR inhibition.

3. Methods

3.1. Cell cultures

Most experiments were performed on primary rat VSMCs, isolated from the aorta thoracalis of 40-60-day-old male Wistar rats. Animals were purchased from Charles River Laboratories and housed at Semmelweis University, Budapest. All animal procedures were approved by the Animal Care Committee of the Semmelweis University, Budapest and the relevant Hungarian authorities (No. 001/2139-4/2012) and adhered to legal and institutional guidelines for animal care. The investigation compiles with the Guide for the Care and Use of Laboratory Animals (NIH, 8th edition, 2011). Animals were sacrificed by decapitation, then the aorta thoracalis was removed and cleaned from tissue remains. The vessels were cut into 1-2 mm wide sections, then incubated with collagenase. The digested sections were placed on a sterile plate in DMEM medium (Biosera, Nuaille, France) completed with 10% FBS (Biosera, Nuaille, France), 1% Glutamax (Gibco, Dublin, Ireland), and 1% penicillin-streptomycin (Lonza, Gampel, Switzerland). Experiments were performed using cells at passage 3.

Experiments involving LMCD1 overexpression were performed on A7r5, an immortalized rat vascular smooth muscle cell line. Lentiviral particles were produced using HEK293T cells.

3.2. Plasmid constructs

3.2.1. LMCD1 Overexpressing plasmid constructs

For LMCD1 overexpression, a pcDNA3.1 plasmid was used, that was designed to express HA-tagged LMCD1 protein. To enhance the complete open reading frame of LMCD1, cDNA obtained from vascular smooth muscle cells (VSMCs) treated with AngII for 2 hours was utilized as the template. The initial PCR product was separated by electrophoresis, was

purified using the GeneJet Gel Extraction Kit (Thermo Fisher Scientific), and then subjected to a second round of PCR with primers containing restriction enzyme sites.

3.2.2. *EGFR* silencing and lentivirus production

To silence *EGFR* gene, lentiviral constructs were used, expressing *EGFR* specific short hairpin RNA (shRNA). shRNAs are able to silence a specific gene via RNA interference. The antisense (guide) strand of the shRNA directs the RISC complex (RNA-induced silencing complex) to the complementary sequence, causing the degradation or the failure of translation of the complementary mRNA. The expressed shRNA sequences were delivered into the VSMCs through lentiviral particles. Lentiviruses were produced in HEK293T cells. Cells were co-transfected with pLKO.1 puro transfer, pCMV-VSV-G envelope, and pCMV-dR8.2 packing plasmids, using calcium-phosphate precipitation method. For each plate of HEK293T cells, transfer, envelope and packing plasmids were mixed into 450 µl of sterile distilled water, then 50 µl of 2.5 M calcium-chloride solution was added. The mixture was added dropwise to 500 µl of 2x HEPES-buffered solution (42 mM HEPES, 15 mM D-glucose, 1.4 mM Na_2HPO_4, 10 mM KCl, 274 mM NaCl 274 mM, pH 7.1). The final mixture was added dropwise to the cells, then the medium was changed after 6 hours with fresh complete DMEM. 48 hours after the transfection, the lentiviral particle containing supernatant was collected, then it was centrifuged (10 minutes at 3000 *rpm*), then poured into a clean tube. Next, supernatants were filtered through a Merck's sterile Millex syringe driven filter unit (0.22µm) into another sterile tube. The purified lentiviral samples were concentrated using the Lenti-X Concentrator kit (Takara-Bio, Kusatsu, Japan) following the manufacturer's instructions. Viral titer was measured using abm's qPCR lentivirus Titration Kit following the manufacturer's instructions. Samples were stored at -80 ºC.

3.4. Affymetrix GeneChip Assay

Following serum deprivation, vascular smooth muscle cells (VSMCs) were treated with 100 nM AngII for 2 hours at 37 °C, after which the cells were lysed using Trizol reagent. The RNA sample quality was assessed with an Agilent BioAnalyzer RNA Nano lab chip prior to conducting the array experiments. Total RNA extraction and analysis using the Affymetrix Rat Gene 1.0 ST GeneChip Array (Affymetrix, Santa Clara, CA, USA) were carried out by UD-GenoMed Medical Genomic Technologies Ltd., based at the University of Debrecen in Hungary. Hybridization and image scanning followed the protocols established by UD-GenoMed Medical Genomic Technologies Ltd. The microarray experiment was conducted in triplicate. Raw CEL files underwent background correction and normalization using the oligo R package, while differential expression analysis comparing Angiotensin II to the vehicle control was performed using the limma R package. We utilized the PROGENy pathway activity analysis tool to assess the changes in pathway activity by AngII. The calculated PROGENy pathway activity scores were normalized against a null distribution (generated through 10,000 random permutations of gene names) to derive pathway activity z-scores.

3.5. Plasmid transfection

LMCD1 was overexpressed using lipofectamine based transfection. Lipofectamine 2000 transfection reagent was obtained from Thermo Fischer (Waltham, MA, USA). Transfection protocol was performed on A7r5 cells, following the manufacturer's instructions. Approximately 200,000 A7r5 cells were plated on 6-well plates, one day prior to transfection and the cells were used for experiments 2 days after the transfection.

3.6. Lentiviral infection

Approximately 200,000-250,000 VSMCs were seeded onto 6-well plates one day prior to infection. Equal titers of lentiviral preparations were diluted in complete DMEM, containing 1% Glutamax and 8 μg/ml PolyBrene (Sigma Aldrich). The cell culture medium was replaced 24 hours later. Cells were subjected to experiment 48 hours after the lentiviral infection.

3.7. Stimulation and pharmacological inhibitor treatments

VSMCs (200,000-250,000/well) were serum-deprived overnight or for 16-48 hours, depending on the experimental requirements. In case of time kinetics examinations, cells were stimulated for varying durations. For agonist stimulations, the following concentrations were used: 100 nM AngII, 50 μg/ml EGF, 1uM AVP, or 3 μM TRV120023. In case of experiments performing inhibitor pretreatments, cells were incubated with serum-free DMEM containing the recommended concentration of the specific pharmacological inhibitor for 10-30 minutes before the hormonal stimulation. In these experiments, 2-hour-long agonist simulations were performed.

3.8. Gene expression measurements

All gene expression measurements were done with real-time quantitative PCR method. After treatment, RNA was isolated from the cells with RNeasy Plus Mini kit from Qiagen (Hilden, Germany) following the manufacturer's instructions. Then the RNA concentrations were measured with the spectrophotometric method, using NanoDrop OneC (ThermoFischer Scientific, Waltham, MA, USA). cDNA was synthesized from the mRNA using RevertAid Reverse Transcription Kit according to the manufacturer's instructions (ThermoFisher Scientific).

For real-time quantitative PCR measurements LightCycler 480 SYBR Green I Master kit (Roche, Basel, Switzerland) was used. To determine the expression levels of

the examined genes, relative quantification was applied and the normalization was against house-keeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). For measuring fluorescent data, LightCycler 480 system (Roche) was used. qRT-PCR primers were obtained from Sigma-Aldrich, their sequences are listed in Table 1. The following thermal cycling protocol was performed: 5 minutes pre-incubation at 95°C, followed by 45 cycles of amplification (10 seconds at 95°C, 5 seconds at 62°C and 15 seconds at 72°C), then a melting curve, that starts at 95 °C for 5 seconds, then 1 minute at 65 °C and 97 °C and cooling 30 seconds at 40 °C. The following equation was used to calculate fold ratios of gene expressions: Ratio = $E^{\Delta Ct\ target\ gene}/E^{\Delta Ct\ GAPDH}$.

3.9. Immunostaining

Cells were seeded into 8-well ibidi plates for immunostaining (10,000 cells/well), then treatment protocols were performed. After treatments, cells were fixated with 4% paraformaldehyde solution in PBS. After fixation, cells were treated with 0.1% Triton X-100 solution (in PBS) for permeabilization, and autofluorescence was reduced by incubation in 0.1% sodium-borohydride. Cells were blocked in 5% BSA in PBS, then stained with primary and fluorescent secondary antibodies. Nuclei were stained with To-Pro reagent (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions. Imaging was performed using Zeiss LSM 710 confocal microscope (Zeiss AG, Jena, Germany), and analyzed with ImageJ software 1.53e.

3.10. Western blot assay

Western blot assays were performed to measure protein expressions. Cells were lysed in Laemmli SDS sample buffer, completed with protease and phosphatase inhibitors, then prepared with sonication. The samples were loaded onto

SDS-polyacrylamide gels for electrophoresis, then transferred to PVDF membranes using the Trans-Blot Turbo Transfer System by Bio-Rad Laboratories (Hercules, CA, USA). Membranes were blocked in PBST containing 5% non-fat milk, then incubated with primary, then secondary antibodies. Secondary antibodies were either fluorescent or HRP-linked. In case of HRP-linked secondary antibodies, ECL chemiluminescent substrate reagents were used for signal detection (Immobilion Western HRP substrate reagent, Millipore, Billerica, MA). Both chemiluminescent and fluorescent signals were detected using Azure c600 system (Azure Biosystems, Dublin, CA). The intensities were quantified by densitometry with ImageJ software 1.53e.

3.11. ^{3}H-Leucine incorporation assay

To determine cellular growth, ^{3}H-leucine incorporation assay was performed. A7r5 cells were plated on a 24-well plate (20,000-30,000 cells/well), then transfected with either LMCD1 overexpressing pcDNA plasmids or empty pcDNA plasmids (control group). One day after the transfection, cells were treated with ^{3}H-labeled leucine containing serum-free DMEM solution. After 24 hours, cells were washed twice with ice-cold PBS and treated with 5% trichloroacetic acid (TCA) for 30 minutes. The TCA was removed, and the cells were washed twice with PBS, and 0.5 ml 0.5 M NaOH was added to the wells and incubated at room temperature for 30 minutes. Samples were collected into 10 ml of OptiPhase HiSafe 3 scintillation cocktail (PerkinElmer, Inc; Waltham, MA, USA). Welles were further rinsed with additional 100 µl of distilled water, which was also added to the scintillation vials. Radioactivity of the samples were measured using Packard Tri-carb Liquid scintillation Counter 2500 TR scintillation reader, alongside a blank control sample containing only distilled water in the scintillation cocktail.

3.12. Wound-healing assay

Migration capability of the cells were assessed with wound-healing assay. A7r5 cells were plated into 3 cm-diameter plates, then transfected with either LMCD1 overexpressing or empty pcDNA plasmids. The cell monolayer was scraped with a sterile 200 µl pipette tip on the following day. The wounds were photographed with Leica DMI6000B (Leica, Wetzlar, Germany) microscope at 5x magnification. 48 hours after the scratching, the wounds were photographed again at the identical positions. The areas of the photographed wounds were measured with ImageJ software.

3. 13. Statistical analysis

Statistical analysis was performed with the help of GraphPad Prism 6, 8 and 9 softwares. In case of real-time q-PCR measurements, performing samples treated with agonist stimulation and also pretreated with pharmacological inhibitors or infected with lentiviral particles were evaluated using multiple linear regression. One-way ANOVA test was performed for the evaluation of real-time q-PCR measurements done on samples that only received agonist stimulation or only virus infection. For the statistical analysis of ^{3}H-leucine incorporation assay and wound-healing assay, paired t-test was used.

4. Results

4.1 Gene expression changes in VSMCs to AngII stimulation

Affymetrix gene-chip assay was performed on primary rat VSMCs, stimulated with 100nM AngII for 2 hours. 74 genes were identified to be upregulated by the hormonal stimuli. *LMCD1* was found to be among the most significantly upregulated genes.

4.2 AngII-mediated upregulation of *LMCD1* in VSMCs

4.2.1. Time kinetics of LMCD1 gene and protein expression in response to AngII

Time dependence of AngII mediated *LMCD1* mRNA upregulation was investigated performing real-time quantitative PCR. Results claim that mRNA levels of *LMCD1* gene peak between 1 to 3 hours after 100nM AngII stimulation, then start to decrease after 4 hours.

To measure the time kinetics of AngII mediated upregulation of LMCD1 protein, Western blot assay and immunofluorescent microscopy was used. Both methods established that LMCD1 protein level peaks 24 hours after the 100nM AngII stimulation.

4.2.2. Intracellular localization of LMCD1

Using immunofluorescent microscopy, significant co-localization with the cis- and trans-Golgi apparatus and also with the nucleus was revealed, utilizing specific markers for these cellular organelles. The nuclear localization of the protein can be explained with its role as a transcriptional cofactor. In the literature, there is no mentioning of LMCD1 to be localized in the Golgi-apparatus. We haven't done further examination whether it has a function there or whether it is only just stored in the cellular organelle.

4.2.3. Role of AT1R and G-protein coupling in AngII mediated LMCD1 expression

Pretreating primary rat VSMCs with 10 μM Candesartan (specific AT1R inhibitor) lead to a significant decrease in AngII mediated *LMCD1* upregulation. Performing $G_{q/11}$ protein inhibition with YM-254890, AngII mediated increase of *LMCD1* mRNA level was also abolished. These result suggest that AngII mediated *LMCD1* upregulation happens via AT1R activation and $G_{q/11}$ coupling. In addition, *LMCD1* was found to be upregulated by arginine-vasopressin too, which hormone exerts its effects on VSMCs mostly by the $G_{q/11}$ coupling of V1 vasopressin receptor.

Using using β-arrestin biased AT1R agonist TRV120023, no LMCD1 upregulation was observed, proving that β-arrestin pathway has no role in the examined process.

4.2.4. Role of secondary messengers in the upregulation of *LMCD1*

Removing calcium from the system with the help of Ca^{2+} chelator 50 μM BAPTA-AM completely inhibited AngII mediated *LMCD1* upregulation in VSMCs, proving that Ca^{2+} signal has an essential role in the process. Meanwhile, inhibiting Ca^{2+}/calmodulin dependent protein kinase II (CaMKII) with 50 μM CK-59, AngII induced LMCD1 upregulation was significantly decreased, but not as robustly as in the case of Ca^{2+} chelation, suggesting that CAMKII has a role, but not an executive role in the process. Inhibition of PKC with 1 μM RO31-8425 and inhibition of FAK and Pyk2 pathway with 1 μM PF-562271 did not show significant changes, so PKC, FAK and Pyk2 activation do not seem to play a role in AngII mediated LMCD1 upregulation.

4.2.5. Role of MAPKs in AngII-mediated *LMCD1* upregulation

As the activation of the MAPK cascades is well-known to play an essential role in the AngII mediated gene expression changes, we performed pretreatment with inhibitors of the three most important MAPK pathways on VSMCs (20 μM

MEK inhibitor PD-98059; 25 μM of JNK inhibitor SP-600125; 50 μM p38 MAPK inhibitor SB-202190). Only the inhibiton of p38 could significantly reduce the AngII mediated *LMCD1* upregulation that reflects to the crucial role of p38 pathway in the process.

As AngII dependent p38 activation is often related to reactive oxygen species (ROS) production, via NADPH-oxidase (NOX) activation, we performed pretreatment with 5 μMs of non-selective NOX inhibitor, diphenyleneiodonium. The pretreatment did not have any significant effect on AngII induced *LMCD1* upregulation which excludes the role of ROS produltion in the examined process.

4.3. Investigation of functional role of LMCD1 in vascular smooth muscle cells

4.3.1. Effect of LMCD1 on cellular proliferation and migration

In order to investigate the role of LMCD1 in the cellular functions, we overexpressed the protein via lipofectamine mediated plasmid transfection in A7r5 rat vascular smooth muscle cell line. Averagely, 15-20% of the cells were successfully transfected.

Cellular proliferation was examined performing 3H-leucine incorporation assay. Transfected cells showed approximately 15% more intense incorporation of radioactively labeled leucine in average, even with a low transfection rate, which concludes that LMCD1 overexpression significantly increases protein synthesis and cellular proliferation.

Wound-healing assay was utilized to measure to examine the effect of LMCD1 overexpression on cellular migration. In control cells, wound areas only reduced by 35% in 48 hours, while in LMCD1 overexpressing groups wound areas decreased decreased by approximately 50% in 48 hours.

4.4. Importance of EGFR transactivation in AngII mediated gene expression changes

4.4.1. Identification of additional gene targets

With Affymetriy gene chip assay, we identified numerous other genes, that are also significantly upregulated by AngII in VSMCs. The genes of four different DUSP isoforms, *DUSP4*, *DUSP5*, *DUSP6* and *DUSP10* were among the most intensely upregulated ones. Among these, we chose *DUSP5*, *DUSP6* and *DUSP10*, each present a different subtype of DUSP proteins, for further investigations.

We also performed pathway analysis on the data, revealing that MAPK and EGFR pathways show the highest activity to AngII stimuli, therefore they potentionally have the most important role in the formation of the long-term effects of AngII.

4.4.2. Lentiviral silencing of *EGFR*

We created two different lentiviral constructs expressing EGFR specific short-hairpin RNAs (shRNA) to silence *EGFR* transcription. Both construct could significantly reduce *EGFR* expression. We chose the more effective one for our further experiments.

4.4.3. Comparing pharmacological inhibition and genetic silencing of EGFR

4.4.3.1. Effect of pharmacological inhibition of EGFR transactivation on AngII-mediated upregulation of DUSP isoforms

Pretreating VSMCs with two different pharmacological inhibitors EGFR (1 μM AG1478, 2.5 μM gefitinib) We could see a robust decrease of AngII mediated upregulation in the case of all the three *DUSP* isoforms which data would prove the essential role of EGFR transactivation in the process.

4.4.3.2. Effect of shRNA mediated silencing of EGFR on AngII-induced upregulation of DUSP isoforms

In order to silence *EGFR,* 48 hour-long lentiviral infection was performed. Interestingly, genetical silencing of *EGFR* could not decrease the AngII mediated upregulation of the DUSP isoforms, as the pharmacological inhibition, only in the case of *DUSP5* we got significant changes. These results question the importance of EGFR transactivation in AngII induced gene expression changes.

4.4.4. Potential pathways beside EGFR-transactivation

As in case that EGFR transactivation is not that important in forming the long-term effects of AngII as we thought before, there must be other signaling pathways who have a more important role.

We did experiments on VSMCs with 1 μM dasatinib pretreatment which provided us interesting data. Dasatinib is a tyrosine kinase inhibitor, targeting BCR/Abl, Src kinases, c-kit and ephrin receptor which is used in the clinical practice in the treatment of different types of leukaemias. Dasatinib pretreatment could reduce significantly the AngII mediated upregulation in the case of all the three examined DUSP isoforms. These results suggest that one of more dasatinib-sensitive tyrosine kinases have to be involved in the process, but their exact identification belongs to future studies.

5. Conclusions

1. *LMCD1* is significantly upregulated by AngII in vascular smooth muscle cells. Its mRNA level peaks 1 to 3 hours after AngII stimulation, while the LMCD1 protein level reaches its maximum approximately 24 hours after AngII treatment.
2. In VSMCs, LMCD1 is mainly localized in the nucleus and shows significant accumulation in the Golgi apparatus after AngII stimulation.
3. AngII induces *LMCD1* upregulation activating AT1R, via $G_{q/11}$ induced calcium signal and p38 MAPK activation.
4. LMCD1 overexpression leads to increased proliferation and migration in vascular smooth muscle cells.
5. MAPK cascade activation and EGFR transactivation seems to play crucial roles in AngII-induced gene expression changes in VSMCs.
6. Genetic silencing of *EGFR* is significantly less effective in AngII-mediated upregulation of certain genes compared to pharmacological inhibition.
7. It is possible that, in addition to EGFR, other tyrosine kinases, such as Src kinase family play key roles in AngII-mediated gene expression changes.

6. Bibliography of candidate's publications

6.1. Publications relevant to the dissertation

I. **Gém, Janka Borbála** ; Kovács, Kinga Bernadett ; Szalai, Laura ; Szakadáti, Gyöngyi ; Porkoláb, Edit ; Szalai, Bence ; Turu, Gábor ; Tóth, András Dávid ; Szekeres, Mária ; Hunyady, László ; Balla, András. Characterization of Type 1 Angiotensin II Receptor Activation Induced Dual-Specificity MAPK Phosphatase Gene Expression Changes in Rat Vascular Smooth Muscle Cells , Cells, 2021 Dec 15;10(12):3538. **IF(2021): 7.666**

II. **Gém, Janka Borbála**; Kovács, Kinga Bernadett; Barsi, Szilvia; Hadadnejadtehrani, Saba; Damouni, Amir; Turu, Gábor; Tóth, András Dávid; Várnai, Péter; Hunyady, László; Balla, András. Role of LMCD1 in the Long-Term Effects of Angiotensin II in Vascular Smooth Muscle Cells, International Journal of Molecular Sciences, 2025 Apr 25;26(9):4053, **IF(2024): 4,9**

Cumulative impact factor: 12,566

6.2. Publications unrelated to the dissertation

I. Kovács, Kinga Bernadett ; Szalai, Laura ; Szabó, Pál **; Gém, Janka Borbála** ; Barsi, Szilvia ; Szalai, Bence ; Perey-Simon, Bernadett ; Turu, Gábor ; Tóth, András Dávid ; Várnai, Péter ; Hunyady, László ; Balla, András. An Unexpected Enzyme in Vascular Smooth Muscle Cells: Angiotensin II Upregulates Cholesterol-25-Hydroxylase Gene Expression ; International Journal of Molecular Sciences, 2023 Feb 16;24(4):3968.. **IF(2023): 4,9**

II. Vass, Zsolt; Shenker-Horváth, Kinga; Bányai, Bálint; Vető, Kinga Nóra, Török, Viktória**; Gém, Janka Borbála**; Nádasy, György L; Kovács, Kinga Bernadett;

Horváth, Eszter Mária; Jakus, Zoltán; Hunyady, László; Szekeres, Mária; Dörnyei, Gabriella. Investigating the Role of Cannabioid Type 1 Receptors in Vascular Function and Remodeling in Hypercholesterolemic Mouse Model with Low-Density Lipoprotein-Cannabioid Type 1 Receptor Double Knockout Animals. International Journal of Molecular Sciences, 2024 Sep 2;25(17):9537. **IF(2023): 4,9**

Cumulative impact factor of all publications: 22,366

Cumulative impact factor of first author and shared first author publications: 12,566