II-01.

Regional expression and structural characterization of P2X7 receptor isoforms in the murine brain

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Keywords: P2RX7, isoform expression, mouse brain

The P2X7 purinergic receptor is an ATP-gated ion channel that plays a crucial role in regulating neuroinflammatory responses and the pathogenesis of neurodegenerative diseases. In rodents, five alternative splice variants of the P2X7 gene have been described (P2rx7a–d and P2rx7k). These include the full-length isoforms A and K, as well as C-terminally truncated variants.

This study aimed to evaluate P2X7 receptor protein levels and isoform expression profiles in selected brain regions of male C57BL/6 and BALB/c mice and female C57BL/6 mice. Transcript and protein expression were analyzed using real-time PCR, semi-quantitative RT-PCR, and Western blotting. Structural differences and agonist-binding site topography were assessed by homology modeling with SWISS-MODEL using UniProt and PDB data. The models were validated with Verify3D and visualized in UCSF Chimera.

Real-time PCR showed that P2X7 gene expression was the highest in the midbrain and cerebellum of C57BL/6 mice. Semi-quantitative RT-PCR confirmed the presence of transcripts encoding isoforms A and C in both C57BL/6 and BALB/c mice. Western blotting showed higher levels of isoform A protein in the midbrain and cerebellum of C57BL/6 mice, whereas isoform C protein levels did not differ across the brain regions examined. No statistically significant differences in the protein levels of either isoform were detected between C57BL/6 and BALB/c mice or between male and female C57BL/6 mice. Females nevertheless showed a slight trend toward higher levels of both isoforms in the hippocampus, midbrain, and cortex. Homology modeling indicated that the topography of the ATP-binding site was conserved across the modeled isoforms despite differences in C-terminal length.

In summary, P2X7 expression showed a regional pattern, with the highest levels in the midbrain and cerebellum largely associated with isoform A. Protein levels of the isoforms examined did not differ significantly by strain or sex, and their modeled ATP-binding sites retained a conserved topography despite differences in C-terminal length. These findings establish a baseline for investigating how regional P2X7 isoform expression relates to receptor signaling in the brain.

This study was funded by the National Science Centre (http://www.ncn.gov.pl), Grant 2023/50/E/NZ4/00550, for A.W.

II-02.

Huntingtin dysfunction during early embryonic development: implications for Huntington's disease pathogenesis

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Keywords: huntingtin, embryonic development, NF-κB signaling

Huntington's disease (HD) is a rare autosomal dominant neurodegenerative disorder caused by an expanded CAG repeat in the HTT gene, resulting in an elongated polyglutamine tract at the N-terminus of the huntingtin protein. Although HD is classically characterized by progressive dysfunction of the basal ganglia and late-onset motor symptoms, increasing evidence suggests that disease-related alterations may begin decades before clinical onset. Moreover, complete loss of huntingtin is embryonically lethal, with developmental arrest occurring around gastrulation, indicating that huntingtin plays essential roles during early development. However, how mutant huntingtin affects embryogenesis and whether these early alterations may contribute to HD pathogenesis remain poorly understood.

To investigate the role of huntingtin during early development, we generated an in vitro model using genetically engineered embryonic stem cells differentiated into embryoid bodies (EBs), which recapitulate key features of early embryogenesis. Fixed EBs were sectioned at 10 µm and analyzed by tissue cytometry. We observed accumulation of mutant huntingtin (Q74) as early as day 3 of EB differentiation, corresponding to a gastrulation-like stage. Mutant huntingtin-expressing EBs showed an approximately four-fold increase in Brachyury-positive cells compared with wild-type EBs, suggesting impaired regulation of Wnt signaling and primitive streak formation. Since these processes are essential for axial patterning and neural tube development, our findings indicate that mutant huntingtin may disrupt early developmental programs relevant to nervous system formation.

In parallel, EBs lacking huntingtin displayed an eight-fold increase in phosphorylated NF-κB-positive cells, suggesting enhanced activation of pro-survival and anti-apoptotic pathways. Consistently, huntingtin-deficient EBs exhibited abnormal compact morphology, lacking the internal cavities required for proper folding and tissue organization. As apoptosis is essential for sculpting the embryo after gastrulation, these findings suggest that loss of huntingtin may impair early morphogenesis by disrupting programmed cell death.

Together, our data provide evidence that huntingtin is critically involved in early embryonic patterning, cell survival, and morphogenesis. These results support the concept that HD may involve developmental components preceding neurodegeneration and suggest that early developmental windows should be considered when designing therapeutic strategies for Huntington's disease.

II-03.

Alpha-synuclein oligomers dysregulate P2X7 receptor expression and function, driving calcium signaling and macrophage activation

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Keywords: alpha-synuclein oligomers (α-SynO), P2X7 receptor, neuroinflammation

Chronic neuroinflammation driven by microglial and macrophage activation is a primary driver of neurodegeneration in synucleinopathies such as Parkinson's disease. Extracellular alpha-synuclein oligomers (α-SynO) act as critical pathological triggers, yet the precise upstream receptor mechanisms mediating their immune effects remain under investigation. The purinergic receptor P2X7 has emerged as a key sensor of cellular stress and inflammation in the central nervous system.

Here, we investigated the impact of α-Syn conformers on P2X7 receptor regulation in murine RAW264.7 macrophages. Intracellular calcium dynamics—reflecting P2X7 channel activation—were monitored using Fluo-4 fluorescence imaging. Changes in P2X7 transcript levels, alongside pro-inflammatory cytokine expression (TNFα, IL-1β, IL-6) and functional phagocytic activity, were evaluated using RT-qPCR and flow cytometry, respectively.

Our data reveal that α-SynO exposure prominently modulates P2X7 receptor expression and directly stimulates rapid intracellular Ca^{2+} influx via functional engagement of the purinergic signaling axis. This P2X7-linked calcium dysregulation coincided with a marked shift in macrophage phagocytic capacity and a robust transcriptional upregulation of downstream pro-inflammatory mediators TNFα and IL-1β. In contrast, monomeric α-Syn failed to elicit comparable receptor activation or inflammatory remodeling.

These findings demonstrate that α-SynO specifically targets and alters the P2X7 receptor pathway, initiating calcium-dependent macrophage activation and neuroinflammatory cascades. Targeting the dysfunctional P2X7 axis may represent a viable therapeutic approach to halt neuroinflammatory feedback loops in synucleinopathies.

This study was funded by the National Science Centre (http://www.ncn.gov.pl), Grant 2023/50/E/NZ4/00550, for A.W.

II-04.

Region-Specific Purinergic Signalling in the Brain of Tsc2 Haploinsufficient Mice

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Keywords: Tsc2 haploinsufficiency, mTOR signalling, purinergic signalling

The mechanistic target of rapamycin (mTOR) is a central regulator of cellular growth, metabolism, and homeostasis, and its dysregulation is implicated in a broad spectrum of neurodevelopmental and neurodegenerative disorders. Mutations in *TSC1/TSC2* result in aberrant mTOR activation and neuronal dysfunction. In our previous studies using the B6;129S4-Tsc2tm1Djk/J mouse model of *Tsc2* haploinsufficiency, we identified brain-region-dependent alterations in cytoskeletal and synaptic proteins, accompanied by ultrastructural abnormalities of microtubules, neurofilaments, synapses, and mitochondria, as well as impaired memory. Building on these findings, the present study examined whether *Tsc2* haploinsufficiency is associated with alterations in purinergic signalling, astrocyte phenotype, and cellular metabolism.

The expression of selected purinergic receptors (P2X7, P2Y6, and P2Y12) and astrocytic markers associated with A1 (*C3*, *Serping1*) and A2 (*Ptx3*, *S100A10*) phenotypes was analysed by qPCR in the cerebral cortex, hippocampus, and cerebellum of two-month-old male *Tsc2*+/− and wild-type mice. In parallel, the activities of lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6PD), and malate dehydrogenase (MDH) were assessed to evaluate potential metabolic alterations.

Purinergic receptor expression showed pronounced brain-region-dependent variation in both genotypes, with significantly higher P2X7 and P2Y12 expression in the cerebellum than in the cerebral cortex and hippocampus in both wild-type and *Tsc2*+/− mice. However, *Tsc2* haploinsufficiency did not significantly affect the expression of P2X7, P2Y6, or P2Y12 in any region examined. No genotype-dependent differences were detected in A1- or A2-associated astrocytic markers or in the activities of LDH, G6PD, and MDH.

Overall, *Tsc2* haploinsufficiency was not associated with detectable alterations in the selected purinergic receptors, astrocytic phenotype markers, or metabolic enzyme activities. In contrast, the consistently higher expression of P2X7 and P2Y12 in the cerebellum across both genotypes indicates pronounced regional specialization of purinergic signalling that is preserved in *Tsc2* haploinsufficiency. These findings highlight distinct regional organization of purinergic signalling that may contribute to differential cellular responses to mTOR dysregulation across brain regions.

II-05.

The role of the DNAJC30 gene in the development of mitochondrial diseases

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Keywords: optic neuropathies, mitochondrial diseases, DNAJC30

DNAJC30, a single-exon nuclear gene encoding a mitochondrial chaperone involved in the turnover of respiratory chain complex I subunits, has recently emerged as an important gene contributing to the spectrum of genetic causes of human optic neuropathies. This is particularly relevant in Central and Eastern Europe, where the autosomal recessive form of Leber hereditary optic neuropathy, known as LHONAR, appears to be more frequent than the mitochondrial form. Besides causing LHON, recessive pathogenic variants in DNAJC30 are a rare cause of severe neuro-muscular mitochondrial disease - Leigh syndrome. This study aims to summarize the current state of knowledge regarding the phenotypes associated with DNAJC30 variants. We have analyzed published clinical cases related to DNAJC30 by reviewing online academic databases, including Google Scholar, PubMed, Mendeley, Science.gov, and ScienceOpen and identified genotype-phenotype correlations. In addition to a thorough literature review, an in-house whole-exome sequencing database was searched for DNAJC30 variants, with variant frequencies compared against gnomAD v4.1 data and pathogenicity prediction tools utilized for novel variants. The analysis confirmed that pathogenic DNAJC30 variants, particularly p.Tyr51Cys, are frequent in Poland, and identified two previously unreported variants predicted to be pathogenic: p.Tyr70Cys and p.Asp208Tyr. Ultimately, these findings may contribute to refining diagnostic pathways and improving genotype-phenotype correlations in mitochondrial optic neuropathies.

II-06.

Effect of SSR504734, a selective glycine transporter type 1 inhibitor, on seizure thresholds and GlyT1 localization in the mouse amygdala kindling model

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Keywords: glycine transporter type 1, epileptogenesis, seizure susceptibility

Introduction: Epilepsy is a chronic neurological disorder characterized by recurrent seizures caused by abnormal electrical activity in the brain. GlyT1 regulates extracellular glycine concentrations and may influence both glycinergic and glutamatergic neurotransmission. This study evaluated the effect of SSR504734, a selective GlyT1 inhibitor, on seizure susceptibility and GlyT1 localization in the mouse amygdala kindling model.

Materials and methods: Fully kindled male CD-1 mice (n=16) received SSR504734 (3, 10, or 30 mg/kg, i.p.) or saline 60 min before determination of the afterdischarge threshold (ADT) and generalized seizure threshold (GST) in a within-subject design. Seizure severity (SS), seizure duration (SD), and afterdischarge duration (ADD) were also assessed; sodium valproate (200 mg/kg) served as a positive control. In a separate protocol, sham and kindled mice received SSR504734 (30 mg/kg, i.p.) or saline daily throughout the 17-session kindling procedure. Double immunofluorescence was used to evaluate GlyT1 localization relative to GFAP in the cerebral cortex, hippocampus, and brainstem and relative to NeuN in the cerebral cortex and hippocampus (n=2-5 per group).

Results: SSR504734 significantly decreased ADT by 20-24% and GST by 17-24% at all tested doses, without affecting SS during either determination. SD was not affected during ADT determination. At 30 mg/kg, SSR504734 significantly increased ADD during ADT determination and both SD and ADD during GST determination. Sodium valproate significantly increased both thresholds and decreased SS. Immunofluorescence analysis showed regional differences in GlyT1 localization. In the hippocampus, more than 90% of the GlyT1 signal was detected within GFAP-positive areas; this proportion was low in the cerebral cortex and intermediate in the brainstem. GlyT1 signal was also detected within NeuN-positive areas in the cerebral cortex and hippocampus. No consistent changes associated with repeated SSR504734 were observed, while differences related to kindling were most evident in the hippocampus.

Conclusion: Acute SSR504734 administration reduced ADT and GST in fully kindled mice, indicating increased seizure susceptibility. At the highest dose, SSR504734 also prolonged seizure and afterdischarge duration in selected assessments. GlyT1 showed region-dependent localization relative to astrocytic and neuronal markers, without consistent changes following repeated SSR504734 treatment.

II-07.

Region-Specific Dysregulation of the Mitochondrial Stress Response in an Alpha-Synuclein Oligomer-Based Mouse Model of Parkinson's Disease

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Keywords: alpha-synucleinopathy, mitochondrial stress response, oxidative stress

Mitochondrial dysfunction and redox imbalance are central features of Parkinson's disease (PD), yet how mitochondrial stress responses evolve across brain regions during alpha-synuclein pathology remains poorly understood. Here, we investigated the temporal relationship between oxidative stress and mitochondrial stress adaptation in a mouse PD model based on bilateral intrastriatal administration of alpha-synuclein oligomers.

Brain tissue was collected at 30, 90, and 180 days post-injection from the striatum, midbrain, cortex, and hippocampus. Oxidative stress was assessed using DCF fluorescence, DHE-derived superoxide signals, and NADPH oxidase activity. Mitochondrial stress-response and adaptive pathways were evaluated by quantifying CHOP, ATF5, SIRT3, and NRF1 protein levels.

Alpha-synuclein oligomers triggered a pronounced and highly region-dependent oxidative response. The midbrain exhibited the most persistent redox disruption, with elevated ROS production, increased superoxide-related signals, and a shift toward a more oxidized state sustained through 90 days. In contrast, cortical alterations were transient and predominantly confined to 30 days, while the striatum showed no significant changes in the oxidative stress parameters examined.

The mitochondrial stress response was similarly heterogeneous and temporally dynamic. In the midbrain, CHOP was elevated at 180 days, with NRF1 and SIRT3 increasing at 30 and 180 days, indicating substantial remodeling of mitochondrial stress adaptation. ATF5 remained largely unchanged, except for a transient increase in the striatum at 90 days. Notably, SIRT3 was reduced in the striatum at 30 and 180 days, while NRF1 was decreased in the cortex at 30 days and in the striatum at 180 days. The hippocampus displayed a distinct pattern, including transient SIRT3 reduction and NRF1 elevation at 90 days.

Together, these findings demonstrate that alpha-synuclein oligomer pathology does not elicit a uniform mitochondrial stress response, but rather drives region- and time-dependent remodeling of stress adaptation. The midbrain emerged as a vulnerable region, characterized by sustained oxidative burden and persistent reprogramming of adaptive pathways. This suggests that failure to resolve redox stress and maintain mitochondrial proteostatic capacity may contribute to progressive vulnerability of midbrain circuits in alpha-synucleinopathy.

Funding: National Science Centre, Poland, grants OPUS LAP 2020/39/I/NZ4/01031 (AA) and SONATA BIS 2023/50/E/NZ4/00550 (AW).

II-08.

Altered calcium homeostasis and PTEN-related signaling in human iPSC-derived neural progenitor cells from Huntington's disease patients

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Keywords: calcium signaling, neural precursor cell, iPSC

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expanded CAG repeat in the *HTT* gene. Although classically associated with adult-onset striatal neurodegeneration, increasing evidence suggests that mutant huntingtin may also disrupt early neurodevelopment. Because huntingtin is essential for embryonic development and neural maturation, we investigated how mutant huntingtin affects human neural progenitor cell (NPC) biology.

Human induced pluripotent stem cell-derived NPCs (hiPSC-NPCs) from HD patients and healthy donors were evaluated for GABAergic differentiation, calcium homeostasis, cell death, proliferation, and PTEN-associated signaling. NPCs expressed Nestin and SOX2 and were differentiated using two- and three-step protocols, generating cells positive for NeuN, TUJ1, Synaptophysin, and GAD65/67, establishing a human model for studying HD-related neurodevelopmental alterations.

HD-derived NPCs showed altered colony morphology, attachment, and cellular status compared with controls. Flow cytometry revealed trends toward increased SOX2 expression and Ki-67 positivity, suggesting altered progenitor maintenance and proliferation. HD NPCs also exhibited increased Annexin V staining and more propidium iodide-positive detached cells, indicating enhanced cell death. In contrast, activated caspase-3/7 levels were reduced, suggesting involvement of cell-death mechanisms not primarily dependent on classical caspase-3/7-mediated apoptosis.

FLUO-4 calcium imaging revealed impaired spontaneous calcium activity in HD NPCs, characterized by fewer spike-like oscillations and predominance of wave-like patterns, potentially associated with elevated basal calcium levels. Because calcium oscillations regulate neural differentiation and survival, these alterations may disrupt calcium-dependent developmental programs.

We further investigated PTEN, a regulator of PI3K/Akt signaling and phosphoinositide metabolism. Pharmacological PTEN inhibition with VO-OHpic and depletion of endoplasmic reticulum calcium stores with thapsigargin produced greater effects in HD NPCs, including reduced Nestin and SOX2 expression, consistent with induction of differentiation.

Together, these findings indicate that mutant huntingtin alters human neural progenitor biology by disrupting calcium homeostasis, survival, proliferation, and PTEN-associated signaling. They support an early neurodevelopmental component of HD and identify calcium- and PTEN-related pathways as potential mechanisms contributing to disease vulnerability before overt neurodegeneration.

II-09.

STIM1 and STIM2 protect cortical neurons from oxygen-glucose deprivation/ recovery injury

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Keywords:STIM proteins, oxygen-glucose deprivatio, NMDA receptors

Stromal interaction molecules STIM1 and STIM2 regulate intracellular Ca2+ homeostasis and neuronal responses to excitotoxic stress. STIM2 additionally regulates activity-dependent NMDAR trafficking, limiting excessive NMDAR-mediated Ca2+ influx. However, their role in neuronal injury induced by oxygen-glucose deprivation (OGD) and subsequent recovery remains poorly understood.
Primary rat cortical neurons were subjected to 3 h OGD followed by 1, 3 or 24 h recovery. Neurons were transfected with YFP, STIM1 or STIM2 expression constructs 20 h before OGD. Cell injury was assessed by lactate dehydrogenase (LDH) release, and protein levels were analysed by Western blotting using equal amounts of total protein.
OGD followed by recovery (a model of ischemia and reperfusion insult in cellular models) induced substantial neuronal injury, as reflected by increased LDH release. Overexpression of either STIM1 or STIM2 markedly reduced LDH release by approximately 50% throughout the 1–24 h recovery period compared with YFP-transfected controls. In non-transfected neurons, endogenous STIM1 and STIM2 levels remained unchanged immediately after OGD but progressively decreased during recovery. STIM1 levels were reduced by approximately 30%, 40% and 70% after 1, 3 and 24 h post OGD, respectively, while STIM2 decreased by approximately 30%, 60% and 70% at corresponding time points. Interestingly, this loss was not observed in YFP-transfected control neurons, indicating that transfection context may modify the molecular response to OGD/recovery. As expected, STIM1 and STIM2 overexpression maintained elevated levels of the respective proteins during recovery. Total GluN2B levels decreased following OGD and remained low at 24 h post OGD, with no consistent increase following STIM1 or STIM2 overexpression. In parallel, STIM2 overexpression was associated with higher Parkin levels at 24 h recovery, while Beclin-1 levels were relatively preserved, suggesting involvement of mitochondrial quality-control and autophagy-related pathways.
These findings identify STIM1 and STIM2 as neuroprotective regulators of neuronal responses to OGD/recovery. The progressive loss of endogenous STIM proteins during recovery and protection conferred by their overexpression suggest that STIM2-mediated protection, apart from counteracting Ca2+ imbalance, may involve NMDAR trafficking and mitochondrial quality-control pathways.

Supported by statutory funds of the Mossakowski Medical Research Institute (Research Topic No. 29/2026).

II-10.

Detection of Early-Stage Protein Aggregation Linked with Neurological Disorders Using Thioflavin T Lasing Spectroscopy

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Keywords: synuclein protein, lasing spectroscopy, Thioflavin T

Early oligomeric and intermediate states of protein aggregation are considered important in the development of neurodegenerative diseases, yet their detection remains challenging because conventional Thioflavin T (ThT) fluorescence is primarily sensitive to mature β-sheet-rich fibrils. ThT lasing spectroscopy provides a sensitive optical approach for probing early stages of protein aggregation, including transient oligomeric states that produce only weak fluorescence signals.

The method exploits the molecular-rotor properties of ThT and the sensitivity of its optical gain to the local microenvironment, particularly microviscosity and restrictions of intramolecular rotation. In a Fabry-Perot cavity composed of two reflecting mirrors, highly concentrated protein-ThT samples act as the gain medium, enabling stimulated emission to amplify changes in the photophysical state of the dye. Studies of α-synuclein demonstrated that lasing spectroscopy reports changes in the protein microenvironment before the formation of mature β-sheet-rich fibrils. Lasing threshold temporal evolution reflected increasing microviscosity and transient interactions between ThT and early protein assemblies, providing an optical signature of transient oligomeric states.
These results establish ThT lasing spectroscopy as an extension method to conventional fluorescence with a large difference reflected in capability of monitoring protein aggregation from early oligomeric and nucleation stages through protofibril and fibril formation.

II-11.

The study on neuroprotective efficiency of phytochemicals isolated from Pulicaria spp. against cell damage induced by oxidative stress and excitotoxic stimuli in primary cortical neurons

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Keywords: neuroprotection, neurodegenerative diseases, phytochemicals

Over the years, various type of phytochemicals derived from numerous plant species were investigated in respect to their potential neuroprotective action in a wide range of experimental in vitro and in vivo models of neurodegenerative diseases. In the current study, we tested the biosafety and neuroprotective profile of three plant specialized metabolites isolated from the plants of *Pulicaria spp*., one monoterpenoid (Pin3; 8,9-epoxy-10-isobutyryloxythymyl isobutyrate), one sesquiterpenoid (Pin17; (1*S*,9*R*)-14-hydroxycaryophyll-5(6)-en-7-one) and one flavonoid (Pin21; chrysosplenol C) in mouse primary cortical neurons. The cells were exposed to tested phytochemicals alone and in combination with oxidative stress inducer, hydrogen peroxide (H_2O_2) and excitotoxic agent, glutamate (Glu). Pin3 and Pin21 when incubated for 24 h with primary neurons was safe up to 50 mM, but at concentration 100 mM increased cell death as evidenced by cell viability (MTT reduction) and cytotoxicity (LDH release) assays, whereas Pin17 up to 100 mM did not evoke any detrimental effects to neurons. We evidenced neuroprotective effects of Pin21 (1-5 mM) against the H_2O_2-induced cell damage in both biochemical assays, whereas for Pin3 (1 and 5 mM) and Pin17 (5 mM) we noticed some tendency in protection but only in MTT reduction assay. Pin3 and Pin21 (0.1-10 mM), but not Pin17 significantly prevented the cell damage evoked by Glu. These data evidenced differential neuroprotective potency of tested phytochemicals and pointed to Pin21 as the most promising candidate for further investigation since it reduced neuronal cell damage induced by oxidative-stress and excitotoxic stimuli.

Acknowledgement: The study was financed by statutory funds of Maj Institute of Pharmacology PAS.

II-12.

Evaluation of anticancer activity of novel dopamine receptor-related hybrid peptides

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Keywords: Hybrid peptides, Ranatensin, Cancer

Cancer is a major global health challenge, and treatment is often limited by drug resistance, systemic toxicity, and tumor heterogeneity. Developing effective, targeted therapeutic strategies therefore remains a major research priority. Hybrid therapeutics, which combine distinct pharmacologically active components within a single molecule, offer a promising approach to simultaneously modulate multiple mechanisms involved in tumor progression.

This project aimed to develop and evaluate the antitumor activity of newly designed hybrid peptides.

The dopaminergic system influences tumor cell growth, survival, blood vessel formation, and treatment response, highlighting dopamine receptors as promising therapeutic targets. Ranatensin is an innate peptide that binds to dopamine D2 receptors. In this project, novel hybrid peptides incorporating a ranatensin-derived pharmacophore and cell-penetrating sequences, including TAT and its analogs, were designed and evaluated.

Their antitumor activity was tested in vitro with glioblastoma and neuroblastoma cell models. The impact of the hybrid peptides on cell viability was examined at various concentrations and exposure durations. To understand the mechanisms of their antitumor effects, morphological changes and cellular responses to treatment were analyzed. Immunocytochemistry was used with markers for cell proliferation and apoptosis.

The results enabled comparison of the biological activity of the developed hybrid peptides across different tumor models, evaluation of their impact on essential cellular processes, and identification of the most promising compounds for additional preclinical investigation.

II-13.

D-galactose induces convergent hallmarks of neuronal aging and inflammatory activation in hiPSC-derived dopaminergic neurons

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Keywords: aging, dopaminergic neurons, D-galactose

Neuronal aging involves progressive disruption of chromatin organization, mitochondrial homeostasis, and cellular stress responses, leading to reduced neuronal resilience and increased susceptibility to neurodegeneration, including Parkinson disease. These changes are accompanied by persistent low-grade inflammatory signaling that can amplify oxidative and metabolic stress. Human neuronal models reproducing these interconnected processes are therefore valuable for investigating mechanisms linking aging with neurodegenerative disease.

The aim of this study was to establish a D-galactose-induced model of neuronal aging in hiPSC-derived dopaminergic neurons and characterize associated molecular, epigenetic, and inflammation-related alterations.

Dopaminergic neurons differentiated from human-induced pluripotent stem cells were treated with D-galactose at 15 mg per mL for five days. Heterochromatin integrity was assessed by immunofluorescence analysis of HP1gamma and H3K9me3. Cellular senescence, viability, oxidative stress, and mitochondrial function were evaluated using complementary assays. Gene expression analysis covered pathways associated with mitochondrial biogenesis, neuronal phenotype, apoptosis, and inflammatory responses, including PTGS2, IL11, CCN2, TGFB1, and a broader panel of cytokine-related markers.

D-galactose exposure reduced the heterochromatin-associated markers HP1gamma and H3K9me3 and was accompanied by increased oxidative stress and impaired mitochondrial function. Expression of the mitochondrial biogenesis regulators PGC1alpha and NRF1 decreased, whereas the senescence-associated marker P16 increased. Dopaminergic neuronal markers TH and MAP2 were significantly reduced, together with increased expression of the pro-apoptotic gene BAX. In parallel, PTGS2 and IL11 were markedly upregulated, consistent with an inflammation-associated stress response. CCN2 expression decreased, whereas TGFB1 remained unchanged, suggesting selective remodeling of inflammatory and trophic signaling rather than uniform activation of a single pathway.

D-galactose induces an aging-like phenotype in hiPSC-derived dopaminergic neurons characterized by epigenetic disorganization, mitochondrial dysfunction, oxidative stress, senescence-associated changes, loss of dopaminergic markers, and activation of inflammation-associated signaling. These findings support the model as a reductionist platform for studying interconnected cell-intrinsic mechanisms of dopaminergic neuronal aging and their contribution to vulnerability to neurodegenerative processes.

Study supported by MMRI PAS Research Fund grant no. FBW 032/2022

II-14.

Neuroprotective efficacy of natural compounds isolated from Pulicaria spp. in oxidative stress-based cellular models of Parkinson's disease

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Keywords: neuroprotection, SH-SY5Y cells, 6-OHDA

Despite many years of experimental research, no neuroprotective drug has been marketed for neurodegenerative diseases. Plant-derived compounds have long been studied as potential candidates for such therapeutics. In this study, we tested the biosafety and neuroprotective profile of two terpenoids and one polyphenolic compound isolated from plants of *Pulicaria spp*., Pin3 (8,9-epoxy-10-isobutyryloxythymyl isobutyrate), Pin17 (14-

hydroxycaryophyll-5(6)-en-7-one) and Pin21 (chrysosplenol C), in cellular Parkinson's disease model utilizing undifferentiated (UN) and retinoic acid (RA)-differentiated human neuroblastoma SH-SY5Y cells. The cells were exposed to tested phytochemicals alone and in combination with two oxidative stress inducers: hydrogen peroxide (H_2O_2) and 6-hydroxydopamine (6-OHDA). Pin3 when administrated for 24 and 48 h to UN- or RA-SH-SY5Y cells was safe up to 10 mM, but at concentration 50 and 100 mM increased cell death as evidenced by cell viability (MTT reduction) and cytotoxicity (LDH release) assays. Pin17 and Pin21 when administrated for 24 and 48 h to UN-SH-SY5Y cells were safe up to 50 mM, but at concentration 100 mM increased cell death and this concentration was also harmful to RA-SH-SY5Y when given for 48 h. We found neuroprotective effects of Pin3 (0.1-1 mM) against the H_2O_2-induced LDH release in RA- but not in UN-SHSY5Y cells, whereas in the 6-OHDA model of cell damage Pin3 (0.1-1 mM) was protective in both cell phenotypes. However, Pin3 did not change the H_2O_2- or 6-OHDA-induced caspase 3 activity in RA-SH-SY5Y cells. We did not find any protection mediated by Pin21 or Pin17 against H_2O_2-or 6-OHDA-evoked cell death in UN- and RA-SH-SY5Y cells. These data suggest neuroprotective potential of Pin3 in oxidative stress-based cellular models of PD, which should be confirmed in other cellular and animal models.

Acknowledgement: The study was financed by statutory funds of Maj Institute of Pharmacology PAS.

II-15.

Rubellins in Neuroinflammation and Tau Pathology Research: Molecular Assessment of Potential Interactions

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Keywords: tau protein, rubellin, structure-activity relationship

Rubellins are naturally occurring bisanthraquinone metabolites that represent promising molecular scaffolds for investigating tau pathology in neurodegenerative diseases. Given the established association between pathological tau aggregation and neurodegeneration, this study aimed to elucidate the structure-activity relationships of natural rubellins and selected hypothetical derivatives and assess their potential as starting points for the development of tau aggregation inhibitors. An integrated *in silico* workflow was employed, combining cheminformatic and physicochemical characterisation, drug-likeness assessment, absorption, distribution, metabolism, excretion and toxicity (ADMET) prediction, molecular docking, and molecular dynamics (MD) simulations. Particular attention was given to the influence of hydroxylation patterns, carbonyl group positioning, and molecular flexibility on predicted tau-binding affinity and pharmacokinetic properties. Molecular docking identified Rubellin D and 3-hydroxy-pre-rubellin A as particularly promising structures, with predicted binding energies of −6.86 and −7.30 kcal/mol and estimated inhibition constants (Ki) of 9.42 and 4.46 µM, respectively. The latter exhibited a more favourable docking profile than the reference compound daunorubicin (−6.08 kcal/mol; Ki = 35.20 µM). The predicted interactions involved key tau residues, including His268, Asp283, and Asn286. Structure-activity analysis indicated that appropriately positioned hydroxyl groups can strengthen ligand-protein interactions, whereas excessive hydroxylation may compromise binding and predicted pharmacokinetic properties. A 200 ns MD simulation further supported the stability of the Tau-Rubellin B complex, which maintained lower backbone RMSD values (0.22-0.27 nm) than apo tau (0.27-0.29 nm).
Overall, the findings highlight rubellins as valuable scaffolds for the rational design of potential tau aggregation inhibitors and identify structural features that may guide further optimisation. Although predicted limitations in intestinal absorption and blood–brain barrier penetration remain challenges, these results provide a basis for developing derivatives with improved pharmacokinetic properties. Experimental validation is required to confirm their effects on tau aggregation and establish their potential relevance to neuroinflammatory and neurodegenerative processes.

II-16.

Sphingosine kinase 1 as a regulatory node linking BDNF/TrkB signalling and alpha-synuclein toxicity

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Keywords: sphingosine kinase 1, BDNF/TrkB signalling, alpha-synuclein

Sphingosine kinase 1 (SPHK1) may represent a regulatory node linking sphingolipid homeostasis, BDNF/TrkB signalling, and alpha-synuclein (alpha-syn) toxicity. We investigated SPHK1 within the BDNF–alpha-syn axis and examined how alpha-syn overexpression affects this relationship.

Parental SH-SY5Y and alpha-syn-overexpressing SH-SNCA cells, generated by lentiviral transduction, were used, with SH-EGFP cells as controls. SPHK1 was activated with K6PC-5 or inhibited with SK1-I. C2-ceramide induced apoptotic stress, while siponimod and ponesimod modulated S1P receptor signalling. Cellular responses were assessed by Western blotting, flow cytometry, immunocytochemistry, and qPCR, with MTT and PI staining used to assess cell viability.

SPHK1 activation increased BDNF mRNA level and was accompanied by enhanced TrkB and AKT phosphorylation, suggesting modulation of neurotrophic signalling in SH-SY5Y cells. In contrast, SPHK1 inhibition did not affect BDNF mRNA level but transiently increased a higher-molecular-weight (HMW~50 kDa) BDNF-immunoreactive species and reduced the mature BDNF-to-HMW BDNF ratio after 24 h. This effect was not sustained at 72 h. SPHK1 inhibition also increased TrkB and MMP9 gene expression, the latter encoding a protease involved in BDNF processing, while reducing that of p75NTR, a proBDNF-associated pro-apoptotic receptor, suggesting a compensatory shift toward mature BDNF/TrkB signalling.

SPHK1 inhibition increased alpha-syn mRNA and protein levels. SH-SNCA cells showed reduced basal BDNF expression and an attenuated BDNF response to SPHK1 activation compared with controls. Following SPHK1 inhibition, the proportion of BDNF-positive cells increased in SH-SNCA cells, whereas only a non-significant trend was observed in controls. This was not accompanied by a comparable change in per-cell fluorescence intensity or BDNF mRNA, indicating a population-level shift in BDNF immunoreactivity after SPHK1 inhibition in SH-SNCA cells. SK1-I also caused greater cytotoxicity and further increased alpha-syn protein levels in SH-SNCA cells. Under ceramide stress, siponimod and ponesimod attenuated cytotoxicity and increased BDNF expression in all examined cells.

Altogether, these findings support SPHK1 as a positive regulator of BDNF/TrkB signalling and suggest that its inhibition promotes alpha-syn accumulation and cytotoxicity, particularly under alpha-syn overexpression. S1P receptor modulation may protect against ceramide-induced stress, potentially involving BDNF/TrkB signalling.

Funding: Research funded by NCN, 2019/32/C/NZ4/00455.

II-17.

HTT Genotype Shapes Bioenergetic and Proteomic Signatures in Huntington's Disease Models

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Keywords: Huntington's disease, bioenergetics, proteomics

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by CAG repeat expansion in the huntingtin (*HTT*) gene. In addition to progressive neurodegeneration, HD is associated with disturbances in cellular bioenergetics, mitochondrial function and redox homeostasis. However, how these metabolic alterations vary with *HTT* genotype, CAG repeat length and cellular background remains unclear. The aim of this study was to compare bioenergetic, redox and proteomic profiles across striatal HD models and patient-derived fibroblasts representing distinct genetic and clinical backgrounds.

Conditionally immortalized murine striatal progenitor cells representing homozygous mutant, heterozygous mutant and wild-type *Htt* genotypes (STHdhQ111/Q111, STHdhQ7/Q111 and STHdhQ7/Q7) were analysed using HPLC-based nucleotide profiling, Seahorse extracellular flux analysis, reactive oxygen species (ROS) measurements and untargeted LC-MS/MS-based proteomics. Patient-derived fibroblasts from an adult-onset HD donor carrying 40 CAG repeats, a juvenile-onset HD donor carrying 67 CAG repeats, and an unaffected control donor were additionally analysed.

Striatal models showed clear *Htt* genotype-associated differences in bioenergetic homeostasis. Homozygous mutant cells displayed a reduced adenosine triphosphate to adenosine diphosphate (ATP/ADP) ratio and adenylate energy charge relative to heterozygous and wild-type cells, together with alterations in reduced nicotinamide adenine dinucleotide to nicotinamide adenine dinucleotide (NADH/NAD+) balance, oxygen consumption and ROS production. Proteomic profiling revealed significant genotype-dependent differences across all three striatal models, indicating broader molecular remodeling accompanying the metabolic phenotype. In patient-derived fibroblasts, both HD lines showed reduced maximal respiration and spare respiratory capacity compared with the unaffected control, consistent with impaired respiratory reserve. Despite comparable respiratory reserve between the two HD lines, the juvenile-onset line showed the highest ROS signal and, compared with adult-onset HD, lower ATP, ADP, total adenine and guanine nucleotide pools, and NAD+.

These findings demonstrate substantial bioenergetic and molecular heterogeneity across HD cellular models and indicate that *Htt* genotype and patient background influence distinct components of cellular energy and redox homeostasis. Integration of metabolic, mitochondrial and proteomic readouts may help identify convergent mechanisms of cellular vulnerability. Validation in larger patient cohorts and patient-derived neuronal models will be important to establish the reproducibility and disease relevance of these signatures.

II-18.

Nuclear Protein Quality Control in the context of Neurodegeneration

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Keywords: ubiquitin–proteasome system, nuclear proteostasis, neurodegeneration

Neurodegenerative diseases pose a major challenge for society. Among them there are Parkinson's and Huntington's disease which are characterized by the formation of toxic protein aggregates in the cell. The misfolded proteins have been identified both in the nucleus and in the cytoplasm. Interestingly, it has been speculated that these harmful species may be trafficked between the cellular compartments, in particular from the cytoplasm to the nucleus, in order to be degraded. The main cellular pathway responsible for the protein degradation is a ubiquitin-proteasome system (UPS). Remarkably, proteasomes have been identified both in the cytoplasm and the nucleus. However, our understanding of the composition of the UPS in the mammalian nucleus is only fragmentary. Furthermore, our knowledge about which proteins are transferred from the cytoplasm to the nucleus for their degradation needs to be also expanded.

Our first aim is to identify novel components of nuclear ubiquitin-proteasome system. To achieve this goal, we are applying a genome-wide CRISPR-Cas9 Knockout screening method. Using a HeLa reporter line, we are able to detect changes in ubiquitin-proteasome-dependent proteolysis specifically in the nucleus. This allowed us to identify genes which deletion was associated with either enhanced or decreased protein degradation.

The second aim is to identify mechanisms regulating recognition and targeting of cytosolic proteins for degradation by the nuclear ubiquitin-proteasome system in mammalian cells. To achieve this, we apply proximity labelling assay method to map the proteomes in different compartments of the cell. Using the *ATG16* Knockout cell line, we were able to detect proteins being translocated into the nucleus upon autophagy stress. These significantly upregulated proteins will be subjected to validation processes.

II-19.

CD73 as a Novel Regulator of Vitamin B12 Homeostasis in the Nervous System

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Keywords: CD73, vitamin B12, intracellular trafficking

Vitamin B12 (B12) is essential for nervous system function, yet the mechanisms governing its intracellular availability in neural cells remain poorly defined. Ecto 5' nucleotidase- CD73, classically associated with extracellular nucleotide metabolism, is also present on intracellular membranes. Our preliminary observations in CD73 deficient mice indicated alterations in B12 metabolism, prompting us to investigate a potential role for CD73 in intracellular cobalamin homeostasis. We therefore examined the role of CD73 in neural B12 homeostasis, with particular emphasis on glial cells and intracellular trafficking.

CD73 knockout and wild type mice underwent behavioural assessment and analysis of B12 status, related metabolites and B12 dependent enzyme activity in brain and peripheral tissues, with or without eight weeks of B12 supplementation. Primary astrocytes and microglia from CD73 knockout and wild type mice, together with human HMC3 and Svg P12 glial cells, were used to assess B12 uptake and intracellular localization of CD73 relative to proteins involved in cobalamin trafficking. Proteomic profiling, coimmunoprecipitation and molecular modelling were used to investigate potential mechanisms.

CD73 knockout mice showed reduced tissue B12 levels, decreased activity of B12 dependent enzymes, and increased circulating methylmalonic acid and homocysteine. These biochemical abnormalities were accompanied by behavioural alterations and persisted despite B12 supplementation. In glial cells, genetic loss or pharmacological inhibition of CD73 reduced B12 uptake. Proteomic profiling identified alterations in pathways related to cobalamin metabolism and cellular homeostasis. CD73 showed strong colocalization with the lysosomal B12 transporter ABCD4, and coimmunoprecipitation of ABCD4 recovered CD73, consistent with their presence within the same protein complex. Molecular modelling further indicated a plausible interaction interface between the two proteins.

These findings identify a previously unrecognized role for CD73 in intracellular B12 homeostasis in neural cells and support its involvement in lysosomal cobalamin trafficking. CD73 dysfunction may therefore limit intracellular B12 availability despite adequate extracellular supply, providing a potential molecular mechanism for neurological manifestations of functional B12 deficiency.

II-20.

Targeting NAD+ Metabolism in Huntington's Disease: From Molecular Dysfunction to Therapeutic Rescue

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Keywords: Huntington's Disease, NAD+, bioenergetics

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by expansion of a CAG repeat within the *HTT* gene, resulting in expression of huntingtin with an expanded polyglutamine tract. Although progressive neurodegeneration is the defining feature of HD, increasing evidence indicates that the disease involves widespread metabolic and bioenergetic abnormalities affecting both the central nervous system and peripheral tissues. We previously identified nicotinamide adenine dinucleotide (NAD+) depletion in skeletal muscle from HD mice and cellular models, as well as alterations in NAD+ homeostasis in HTT-deficient cardiomyocytes, suggesting that impaired NAD+ metabolism may reflect both toxic effects associated with mutant huntingtin and disruption of physiological huntingtin-dependent processes.

This study investigated alterations in NAD+ metabolism across experimental models and patients with HD and evaluated whether restoration of NAD+ availability could mitigate molecular and functional disease phenotypes.

NAD+ metabolism was analysed in tissues and blood from R6/1 HD mice and C57BL controls, ST Hdh cells carrying expanded CAG repeats, and fibroblasts and blood from HD patients and unaffected controls. NAD+ and related metabolites were quantified using LC-MS and HPLC-based metabolomic approaches. Expression of enzymes involved in NAD+ biosynthesis and consumption was assessed by qPCR. Mitochondrial respiration and oxidative stress were evaluated using Seahorse analysis and ROS measurements. Neurological, cardiac and skeletal muscle phenotypes were assessed by behavioural testing, echocardiography and grip strength. The effects of NAD+ restoration were examined following treatment with nicotinamide riboside (NR).

HD was associated with tissue-specific disturbances in NAD+ metabolism, including alterations consistent with reduced biosynthetic capacity and increased NAD+ consumption. Changes in circulating NAD+ and nicotinamide-related metabolites were also detected in HD patients and showed associations with disease progression. NR improved neurological performance, cardiac function and skeletal muscle strength in HD mice, reduced mutant huntingtin accumulation in the striatum, improved mitochondrial function and attenuated oxidative stress in cellular models.

These findings identify NAD+ metabolic dysregulation as a molecular component of HD pathophysiology affecting both central and peripheral tissues. Restoration of NAD+ availability may therefore represent a therapeutic approach targeting bioenergetic dysfunction across multiple manifestations of HD.

II-21.

Clinical determinants of serum BDNF concentration in Parkinson's disease

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Keywords: Parkinson's disease, brain-derived neurotrophic factor, serum biomarkers

Background: Brain-derived neurotrophic factor (BDNF) plays an important role in neuronal survival, synaptic plasticity, and neuroprotection. In normal physiological conditions, peripheral BDNF cannot cross the blood–brain barrier, while its integrity may be disrupted in Parkinson's disease (PD). Serum BDNF was proposed as a potential PD biomarker, although previous studies reported inconsistent findings.

Objectives: This study aimed to assess clinical determinants of serum BDNF in PD patients.

Materials and methods: This cross-sectional study included 158 patients with PD. Serum BDNF was measured using enzyme-linked immunosorbent assay (ELISA). Motor and non-motor symptoms, cognitive function, depressive symptoms, comorbidities, pharmacotherapy, and laboratory parameters were evaluated. Independent BDNF determinants were assessed using multivariable linear regression.

Results: Median serum BDNF was 56.3 [IQR: 27.6] ng/ml. Women presented higher BDNF concentrations than men (p=0.047). BDNF was positively correlated with Movement Disorder Society-Unified Parkinson's Disease Rating Scale part III (MDS-UPDRS III) OFF scores (r=0.213, p=0.015), with trends for ON scores (r_s=0.146, p=0.095) and Hoehn & Yahr stage (r=0.152, p=0.062). No associations with cognitive status or depressive symptoms were found. In multivariable analysis, higher BDNF was independently associated with MDS-UPDRS III OFF scores (β=0.341, p=0.010) and Catechol-O-methyltransferase (COMT) inhibitor use (β=12.158, p=0.016). Male sex (β=−11.396, p=0.006) and disease duration of 5–10 years (β=−14.382, p=0.019) were associated with lower BDNF.

Conclusions: Serum BDNF has limited utility as an independent PD progression biomarker due to clinical and treatment-related variability. Its association with motor severity may reflect compensatory neuroprotective mechanisms influenced by dopaminergic treatment.

II-22.

Effect of siponimod on spingosine-1-phosphate related signaling in the acute phase of spinal cord injury

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Keywords: spinal cord injury, sphingolipids, siponimod

Spinal cord injury (SCI) is a serious damage to the spinal cord (SC) that triggers a complex pathological mechanisms including neuroinflammation and excitotoxicity. Neuroinflammation that occurs after SCI is a decisive factor for central nervous system (CNS) tissues fate. Understanding this inflammatory cascade is crucial for developing neuroprotective interventions. Sphingosine-1-phosphate (S1P) receptor modulators have emerged as promising multi-target neuroprotective agent in CNS trauma after successful application in multiple sclerosis.

This study aimed to examine the mRNA levels of enzymes and receptors engaged in S1P signalling in the SC of rats 24h after SCI. Moreover, the effect of siponimod (SIPO, S1P receptor (S1PR) 1&5 modulator) was evaluated.

Female Wistar Rats (300g±30g) were divided into the three experimental groups: Control, Compressed (COMP) and Compressed rats treated SIPO (*i.p.* 2mg/kg). Control and COMP animals received only vehicle (DMSO). SCI was induced by compression at Th9 vertebral level. SC was compressed using a 40g device for 15 minutes. SIPO was administered 30 minutes after the compression and 24h later cranial (SCU), caudal (SCD) and site of injury (SCX) of the SC was isolated for qPCR. All experiments were analysed using one-way ANOVA followed by Tukey's post-hoc test (n=4-5 rats/group).

We observed a significant elevation of *Sphk1, Sphk2*, and *S1pr3* gene expression in all analysed parts of COMP SC. At the same time COMP animals *S1pr1* mRNA levels was downregulated in all analysed structures, where *S1pr5* was reduced in SCU and SCX. SIPO administration further upregulated *S1pr3* mRNA levels in both SCD and SCU as well as *Sphk1* in SCU. Moreover, SIPO reduced mRNA levels of *S1pr5* in SCU.

Presented results reveals compensatory response within the sphingolipid signalling network where alteration of *S1pr1* and *-5* stimulates alternative signalling pathways involving *Sphk1* and *S1pr3* axis that have impact on cell survival, astrocyte activation and scar formation. Moreover, combinatorial therapies-potentially coupling SIPO with S1PR3/SPHK1 modulators-may be required to fully suppress secondary neuroinflammation and optimize functional recovery after SCI.

Supported by Polish–Slovak 2025-2026 Joint Project No.10, VEGA 2/0115/24, and MMRI PAS statutory budget theme No.7.

II-23.

α-Synuclein Induces a Selective Transcriptional Response in Monocytic Cells Without Triggering Secondary Microglial Activation

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Keywords: microglia, monocytes

Background: α-Synucleinopathies, including Parkinson's disease, are increasingly recognized as neuroimmune disorders in which pathological α-synuclein (α-syn) acts both as a cytotoxic protein and as a modulator of innate immunity. In addition to activating microglia, extracellular α-syn may recruit peripheral monocytes to the central nervous system and influence their differentiation and inflammatory phenotype. However, whether α-syn-stimulated monocytes propagate inflammatory signalling to resident microglia remains unclear.

Methods: Monocytic cells were exposed to α-syn or lipopolysaccharide (LPS) for 24 h. Cellular activation was assessed by flow cytometric analysis of CD80 and CD68 expression and by measuring the expression of genes associated with inflammatory and regulatory responses. To investigate secondary microglial activation, stimulated monocytic cells were washed to remove residual α-syn or LPS and transferred for an additional 24 h to insert-based cocultures containing previously untreated BV2 microglial cells. This approach allowed the effects mediated by stimulated monocytic cells to be distinguished from the direct activity of the applied stimuli.

Results: Flow cytometric analysis showed that α-syn exposure did not increase the expression of either CD80 or CD68, indicating the absence of a conventional activation phenotype detectable using these markers. Nevertheless, α-syn induced a distinct transcriptional response characterized by increased expression of **IL10, IL6, CSF1,** and **NOS2**, without altering **TNF** expression. In comparison, LPS elicited a broader inflammatory response and increased the expression of **IL10, IL6, CSF1, NOS2,** and **TNF**. Despite the transcriptional changes observed in α-syn-treated monocytic cells, these effects did not translate into detectable secondary activation of BV2 microglia in the insert-based coculture system. By contrast, prior stimulation of monocytic cells with LPS resulted in the activation of cocultured BV2 cells.

Conclusions: α-Syn induces a selective and atypical response in monocytic cells that is detectable at the transcriptional level but is not accompanied by increased CD80 or CD68 expression. Importantly, this response appears insufficient to propagate inflammatory activation to neighbouring microglia under the experimental conditions used. These findings distinguish α-syn-induced monocyte reprogramming from the broader and functionally transmissible response elicited by LPS.

This work was funded by the statutory research funds of the Mossakowski Medical Research Institute, Polish Academy of Sciences (MMRI PAS), grant no. FBW-060.