|A1|

Alterations of extracellular ATP-dependent signal transduction in rodent model of environmentally triggered autism

Babiec L., Wilkaniec A., Adamczyk A.

Department of Cellular Signaling, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Purinergic signaling involves a complex interplay between membrane-localized receptors (purinoceptors, P1 and P2), a family of ectonucleotidases, plasmalemmal channels (pannexins, connexins), and transporters that regulate the extracellular levels of purines, such as adenosine triphosphate (ATP) and adenosine. This system plays a crucial role in synaptic transmission and neuromodulation. Consequently, any deregulation of ATP-dependent signaling can lead to synaptic dysfunction and the development of autism-like behaviors.

In this study, we used a well-established model of autism spectrum disorders (ASD), involving a single prenatal exposure to valproic acid (VPA), to investigate alterations in extracellular ATP-dependent signaling and their association with ASD-like behaviors. Wistar rats were given a single intraperitoneal (i.p.) injection of VPA (450 mg/kg body weight) on gestational day 12.5. The experiments were conducted on cerebrospinal fluid (CSF) and brain tissue from 52-days old male offspring, which received a single i.p. injection of the non-selective P2 purinoceptor antagonists PPADS and isoPPADS (12.5 mg/kg body weight) 24 hours prior to behavioral testing.

The results demonstrated elevated levels of ATP, ADP, and AMP in the CSF of VPA-exposed offspring, along with increased expression of proteins involved in ATP release and P2 receptors (P2X3, P2Y1, and P2Y12) in the cerebral cortex of VPA animals, suggesting significant upregulation of purinergic signaling. Overstimulation of P2 purinergic receptors activated the mTOR kinase and led to dysregulation of presynaptic proteins synaptophysin and synaptobrevin (VAMP1/2), as well as the postsynaptic density protein 95 (PSD-95). Finally, purinergic signaling disturbances may be responsible for the development of autism-like behaviors, as the stereotypical and anxiety-related behaviors observed in animals prenatally exposed to VPA were significantly reduced following treatment with either PPADS or isoPPADS.

In conclusion, alterations in the ATP-dependent signaling cascade could be a potential mechanism driving mTOR kinase overactivation, synaptic dysfunction, and the development of autism-like behaviors. Targeting P2 receptor antagonism may offer a therapeutic approach for ASD treatment.

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|A2|

Assessing the Impact of Hypoxia on Coding and Non-Coding Gene Expression in Glioblastoma Stem-Like Cells

Balakrishnan A.[1], Goryca K.[2], Palatini J.[2], Godlewski J.[3], Bronisz A.[1]

[1]Tumor Microenvironment Laboratory,, Mossakowski Medical Research Institute, Polish Poland
[2]Genomics Core Facility, Center of New Technologies (CeNT), Poland
[3]Department of NeuroOncology, Mossakowski Medical Research Institute, Poland

Immunotherapy's effectiveness against glioblastoma, a brain tumor characterized by diversity and immune-suppressive regions overlapping with low oxygen levels, is limited. While targeting signaling pathways activated in these hypoxic zones holds promise for treatment, it risks disrupting essential cellular functions due to the broad activity of protein-based hypoxia responses like HIF1A, which aren't cancer-specific. However, regulation of HIF1A at the protein level, which stabilizes in hypoxic conditions, contrasts with its antisense, non-protein-coding transcript, HIF1A-AS2, which is transcriptionally upregulated and offers greater specificity for targeting hypoxic gene expression changes. This transcript also serves as a precise marker for identifying hypoxic cancer niches. This study aims to exploit this distinctive regulatory feature by stratifying glioblastoma stem-like cells (GSCs) according to HIF1A-AS2 expression, enabling precise analysis of the tumor's specific responses to hypoxia.

The research aims to investigate how hypoxic conditions influence gene expression in GSCs by studying their global transcriptome. This analysis helps understand the roles of both coding (mRNA) & non-coding (ncRNA) transcripts, offering insights into tumor progression, diversity, and potential resistance to therapy. A cohort of patient-derived GSCs (n=5) was categorized based on HIF1A-AS2 expression levels and cultured under either normoxic (20% O_2) or hypoxic (1% O_2) conditions for 24 hours before extracting RNA. RNA sequencing was utilized to profile both mRNAs and ncRNAs. Differential expression analysis was then conducted to discern the transcriptional differences between the groups. This analysis encompassed a comprehensive evaluation of genome-wide gene signatures, including 13,765 mRNA and 5,083 ncRNA signatures. Techniques such as PCA, Heatmaps, and Volcano plots were employed to visualize and further analyze these differences. Bioinformatics and statistical analysis revealed significant transcriptome changes in all GSCs under hypoxia, with a stronger response in cells expressing HIF1A-AS2. Specifically, while most mRNA levels decreased, ncRNAs showed both increases and decreases, highlighting potential new targets responsive to hypoxia. As hypoxia typically suppresses gene expression, the upregulation of specific ncRNA genes emphasizes their critical role in cellular adaptation to hypoxia.

|A3|

Long-term effectiveness and safety of spermidine for liver, kidney and pancreas function in rat model of Parkinson's disease

Grembecka B., Harackiewicz O., Majkutewicz I., Rucinski J., Wrona D.

Department of Animal and Human Physiology, Faculty of Biology, University of Gdansk, Gdansk, Poland

Objectives: Patients with Parkinson's disease (PD) often exhibit signs and symptoms of peripheral autonomic nervous system dysfunction, which may appear even before motor deficits develop. Alleviating non-motor symptoms of PD remains a significant challenge, and the search for effective therapeutic substances continues. In our previous research, we demonstrated that spermidine (SPER) has immunomodulatory effects on the peripheral immune system. In this study, we assessed the safety profile of long-term SPER administration by evaluating standard markers of liver, kidney, and pancreatic function in a rat model of progressive neurodegeneration.

Method: One day after striatal 6-hydroxydopamine stereotaxic microinjection and continuing for the next 6 months, male Wistar rats received orally 10 mg/kg of SPER or water as control. The peripheral blood was collected via cardiac puncture and plasma samples were analysed using veterinary clinical chemistry analyzer Exigo C200 (comprehensive panel).

Result: A 6-month supplementation of SPR in rats with PD model decreases total bilirubin level while increases amylase and urea concentrations. It is worth highlighting that the changes observed in both the control and experimental groups are consistent with reference values. The levels of other measured biochemical parameters, including blood glucose concentration, did not differ significantly from those of the control groups and remained within the reference range.

Conclusion: Long-term administration of the SPER in 6-hydroxydopamine-induced rat model of PD improves liver function and is safe for kidney and pancreatic function.

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|A4|

PSB-KD107 agonist and PSB-CB5 antagonist of cannabinoid receptor GPR18 improve spatial memory in a streptozotocin-induced Alzheimer's disease rat model

Harackiewicz O.[1], Swiatek G.[1], Grembecka B.[1], Majkutewicz I.[1], Honkisz-Orzechowska E.[2], Kaleta M.[2], Kiec-Kononowicz K.[2], Wrona D.[1]

[1]Department of Animal and Human Physiology, Faculty of Biology, University of Gdansk, Gdansk, Poland
[2]Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy, Jagiellonian University Medical College, Cracow, Poland

Introduction: Cannabinoid-activated orphan G protein-coupled receptor (GPR18) is found on microglia cells, and its activation can modulate microglia-induced neuroinflammation.
Aim: Using PSB-KD107 as GPR18 agonist and PSB-CB5 as antagonist, we investigated the role of GPR18 in attenuating inflammatory processes in a streptozotocin (STZ)-induced rat model of neuroinflammation leading to Alzheimer's disease (AD).

Methods: Two weeks after intracerebroventricular (ICV)-STZ (3 mg/ventricle) or VEH microinjections, Wistar rats were exposed to 7 consecutive day intraperitoneal injections of PSB-KD107 (AD+ PSB-KD107) or solvent (1%Tween) (AD+solvent or VEH+solvent) or PSB-CB5 (AD+PSB-CB5) groups at a dose of 5 mg/kg b.w. and Morris water maze (MWM) test. On the probe day (no platform), spatial memory disorders were measured as the latency to reach the platform (s), total distance swum (cm), and percentage of time spent in the critical quarter (CQ) where the platform was before (%).

Results: Compared to the controls (VEH+solvent), the AD+solvent group showed significantly longer time to reach the platform and total distance swum, as well as lower % time in CQ. After a 7-day injection of a GPR18 agonist or antagonist, longer time in CQ and lower latency were observed in both the AD+PSB-KD107 and AD+PSB-CB5 groups, compared to the AD+solvent group. However, there were no significant differences in measured parameters between AD+PSB-KD107 and AD+PSB-CB5 animals. In addition, after both GPR18 agonist and antagonist injections in the AD model, the latency significantly differed compared to the controls, while no effect on the total distance swum was found.

Conclusions: Both PSB-KD107 (a GPR18 agonist) and PSB-CB5 (a GPR18 antagonist) at a dose of 5 mg/kg b.w. improved spatial reference memory as indicated by prolonged time in CQ and shortened latency in rats with the ICV-STZ model of AD evaluated in MWM. The obtained results increase our knowledge of the potential application of GPR18 in attenuating neuroinflammation.

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|A5|

Immunopeptide Profiling in Glioblastoma: The Therapeutic Potential of Extracellular Vesicles in Enhancing Immune Responses

Kiel K.[1], Maluszek M.[2], Stepnik D.[2], Przybylski M.[3], Gazi O.[3], Chiocca E.[4], Godlewski J.[5], Bronisz A.[1]

[1]Tumor Microenvironment Laboratory, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[2]The Laboratory of Cytometry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
[3]Department of Medical Microbiology, Medical, University of Warsaw, Warsaw, Poland
[4]Department of Neurosurgery, Brigham and Women's Hospital, Harvard Medical School, Boston, USA[5] Department of NeuroOncology, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Immunopeptide-based therapies represent a promising avenue for improving patient outcomes compared to traditional treatments. However, the challenge of identifying cancer-specific antigens in personalized medicine remains. Extracellular vesicles (EVs) offer a promising solution, as studies suggest they contain sufficient amounts of MHC-presenting peptides, making them valuable for cancer immunotherapy development. Our work aims to assess the adequacy of isolation procedures and the optimization of instrumental and analytical parameters to detect and characterize MHC receptor-presenting peptides found in EVs. This will pave the way for developing personalized vaccination strategies to prevent cancer recurrence, inspiring and motivating further research in this field.

We conducted a peptide identification randomized study for ex vivo immunotherapy using glioblastoma stem-like cells (GSCs) from brain cancer patients. To reflect patient biospecimen heterogeneity, a critical factor, cells derived from six glioblastoma patients were cultured ex vivo and infected with a clinically relevant immune modulating HSV1 oncolytic virus (OV, MOI 0.1, 12h) to transform immunosuppressive tumor into an immunostimulatory one. GSCs were separated from the EV and EV-free secretome by ultracentrifugation, providing the material for proteomic and immunopeptidomic analyses.

Our findings revealed significant alterations in EV proteome/peptidome profile upon OV infection. Applying bioinformatic analysis, we identified a cohort of immunopeptides/proteins deregulated upon OV infection (n=879 and 2156, respectively; $p<0.05$, FC>1.5), and functional analysis linked bulk to immune response activation such as leukocyte degranulation and neutrophil/granulocyte activation. Ex vivo cell activation tests demonstrated increased activity in immune cells, particularly NK cells, DCs, NKTs, and CD8 T cells in the presence of EVs derived from OV-infected GSCs.

Our analysis of the GSCs secretome in response to OV therapy offers valuable insights into the immune response complexity in glioblastoma. The immunostimulatory properties of secreted EVs underscore their therapeutic potential, indicating a promising avenue for developing innovative immunotherapeutic strategies in glioblastoma treatment.

|A6|

Analgesic peptide biphalin demonstrates inhibitory effect on tumor growth in a mouse model of colon cancer

Lazarczyk M.[1], Poznanski P.[2], Skiba D.[1], Mickael ME.[1], Nawrocka A.[1], Jaskuła K.[1], Kosson P.[3], Wijas B.[4], Bieńkowska I.[4], Marczak S.[4], Leśniak A.[5], Sacharczuk M.[1,5]

[1]Department of Experimental Genomics, Institute of Genetics and Animal Biotechnology, Polish Academy of Sciences, Poland
2Laboratory of Host-Microbiota Interactions, Nencki Institute of Experimental Biology, Polish Academy of Sciences,, Poland
3Environmental Laboratory of Pharmacology and Toxicology Research, Mossakowski Medical Research Centre, Polish Academy of Sciences, Poland
[4]Institute of Genetics and Animal Biotechnology, Polish Academy of Sciences, Poland
[5]Department of Pharmacotherapy and Pharmaceutical Care, Faculty of Pharmacy, Medical University of Warsaw, Poland

Pain is a non-specific symptom accompanying numerous diseases of distinct body systems and their innervated organs. In advanced stages of cancer, patients experience neuropathic pain difficult to treat. It originates from the entrapment and compression of nerves by the growing primary tumor and metastatic lesions that sometimes even leads to nerve damage. On the other hand, opioid use is limited in clinical practice due to their serious side effects such as dependency or respiratory depression when overdosed. It is widely evidenced that opioids, apart from their modulatory activity on pain transmission, are involved in intracellular mitogenic pathways and may stimulate or inhibit the proliferation of normal or cancer cells. Many animal and clinical studies indicate that the effect of morphine on tumor growth could be both beneficial or adverse, depending on various factors such as cancer type and stage, treatment regimen, etc. An ideal opioid drug should both alleviate cancer pain and suppress tumor growth. Biphalin is a dimeric enkephalin analogue, synthesized for the first time by Professor Andrzej Lipkowski, that displays affinity to all opioid receptor types. Throughout the last few decades biphalin has been extensively studied mainly as a candidate for pain treatment, including neuropathic or inflammatory pain. Here, we present the results from our in vivo study that expands the knowledge on the anticancer properties of biphalin that seem to be superior to morphine. Biphalin (0.714 mg/kg) administered daily via subcutaneous (sc.) injections, inhibited tumor growth in BALB/c mice inoculated sc. with murine colon cancer CT26 cells, while an equal dose of morphine sulphate stimulated tumor growth. On the contrary, in a study with C57BL/6J mice inoculated sc. with mouse melanoma B16F0 cells, neither biphalin or morphine (0.714 mg/kg) had any effect on tumor growth. Interestingly, both tested opioids increased proliferation/viability, particularly of CT26 cells in vitro, that suggests the involvement of additional systems in the inhibitory effect of biphalin seen in vivo, that are absent in cell culture. Further studies are required to establish the mechanism of anti-cancer properties of biphalin in vivo.

|A7|

The inhibition of bromodomain and extraterminal (BET) proteins protects against microglia-mediated neuronal loss in vitro

Matuszewska M., Wilkaniec A., Cieslik M., Czapski G.

Department of Cellular Signalling, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

The appropriate functioning of the immune system during disease is beneficial; however, its prolonged, excessive, or extensive activity is detrimental. Given that neuroinflammation is a component of all neurodegenerative disorders, including Alzheimer's disease (AD), attenuation of inflammatory processes may be a viable strategy for neuroprotection. Bromodomain and extraterminal domain (BET) proteins, which are readers of the histone acetylation code, collaborate with transcription factors to regulate gene transcription. Inhibitors of BET proteins have effectively mitigated inflammation-related signaling in macrophages; however, their role in microglial cell activation remains poorly understood. This study aims to investigate whether inhibition of BET protein can prevent microglia-dependent neuronal cell loss in vitro.

In our study, we treated murine microglial BV2 cells with bacterial lipopolysaccharide (LPS) or Amyloid-β (Aβ) and analysed the impact of this stimulation on murine neuronal HT22 cells.

Our results indicate that among the brain-resident BET isoforms, only Brd4 is upregulated in microglial BV2 cells during pro-inflammatory stimulation. Treatment with JQ1, a pan-inhibitor of BET proteins, attenuated the LPS-induced upregulation of mRNA levels for pro-inflammatory genes such as Il1b, Il6, and Tnf in BV2 cells. Furthermore, pre-treatment of BV2 cells with JQ1 mitigated the cytotoxic effects of LPS-stimulated BV2 cells on HT22 cells. Then, we observed that among the tested forms of Aβ (oligomers, protofibrils, and fibrils), only fibrils were capable of inducing activation of microglial BV2 cells. The conditioned medium from Aβ-stimulated BV2 cells elicited a cytotoxic effect on HT22 cells. Notably, the viability of neuronal cells was preserved when BV2 cells were pre-treated with JQ1. Similarly, experiments conducted using co-culture inserts demonstrated that inhibition of BET proteins in BV2 cells reduces their neurotoxic potential.

Our results demonstrated that inhibition of BET proteins may be considered a potential neuroprotective strategy during neuroinflammation.

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|A8|

The effect of sphingosine kinase 1 pharmacological modulation on brain-derived neurotrophic factor (BDNF), Bcl-2 family members and pro-survival AKT and ERK1/2 kinases in alpha-synuclein transduced cells. The promising role of siponimod and ponesimod

Motyl J.[1], Czubowicz K.[2], Wencel A.[1], Strosznajder R.[2]

[1]Laboratory of Tissue Engineering, Hybrid and Analytical Microbiosystems Department, Nalecz Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences, Warsaw, Poland
[2]Laboratory of Preclinical Research and Environmental Agents, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

The interaction between sphingolipid imbalance and alpha-synuclein (α-syn), a protein mutated/overexpressed in Parkinson's disease (PD), is still poorly explored. Here we analysed the role of sphingolipids disturbances, evoked by pro-apoptotic cell-permeable C2-ceramide, the pharmacological modulation of sphingosine kinase 1 (Sphk1), synthesizing pro-survival sphingosine-1- phosphate (S1P), as well as by direct modulation of S1P receptors on the expression/protein level/activity of critical proteins in cell survival/death mechanism. The SH-SY5Y cells with the lentiviral-mediated transfer of human α-syn gene – SH-SNCA and empty vector transduced cells, as an appropriate control represented PD cellular model.

PCR-RT analysis indicated, that α-syn overexpression reduced the mRNA level of brain-derived neurotrophic factor (BDNF) and increased the pro-apoptotic/anti-apoptotic Bax/Bcl-2 ratio. Furthermore, treatment with Sphk1 inhibitor (SK1-I) and C2-ceramide further increased the expression of α-syn in SH-SNCA cells, both at mRNA and protein level, which was assessed by PCR-RT and Fluorescence-Activated Cell Sorting (FACS) technique, respectively. At the same time, simultaneous incubation with SK1-I and C2-ceramide was highly toxic, inducing death in almost all studied populations. Moreover, Sphk1 activation and the management of SK1-I/C2-ceramide exert opposite effects on the Bax/Bcl-2 ratio, inducing its reduction and elevation, respectively. On the other hand, BDNF mRNA level was up-regulated by the Sphk1 activator and down-regulated by C2-ceramide. Contrary, the expression of neurotrophic tyrosine kinase receptor 2 (NTRK2), being a BDNF receptor, was declined by the Sphk1 activator and up-regulated by SK1-I and C2-ceramide treatment.

Finally, the administration of S1P receptor modulators, such as siponimod and ponesimod abolished reduced by C2-ceramide BDNF expression and enhanced lessened phosphorylation crucial for the activity of pro-survival AKT and ERK1/2 kinases. Surprisingly, ponesimod also increased the Bax/Bcl-2 ratio. Finally, both compounds markedly protected cells against C2-ceramide toxicity.

Our results suggest that the imbalance in sphingolipid ratio in favour of pro-apoptotic ceramide may be associated with α-syn overexpression, Bax/Bcl-2 elevation, BDNF reduction, as well as AKT/ERK1/2 kinases inhibition. On top of that, siponimod and ponesimod offer protection against the above alterations, making them promising candidates for future PD studies.

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|A9|

The effect of siponimod on alpha-synuclein, glial cells protein markers and brain-derived neurotrophic factor (BDNF) in rats spinal cord injury model

Motyl J.[1], Wencel P.[1], Kisucka A[3]., Czubowicz K.[2], Lukacova N[3], Strosznajder R.[2]

[1]Laboratory of Tissue Engineering, Hybrid and Analytical Microbiosystems Department, Nalecz Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences, Warsaw, Poland
[2]Laboratory of Preclinical Research and Environmental Agents, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[3]Institute of Neurobiology of Biomedical Research Centre of Slovak Academy of Sciences, Kosice, Slovakia

Siponimod, currently used in Multiple Sclerosis, seems to be a promising candidate for spinal cord injury (SCI) management. It prevents lymphocyte infiltration into the brain and modulates neuronal and glial cell sphingosine-1-phosphate (S1P) receptors, activating the pro-survival mechanism, among other brain-derived neurotrophic factor (BDNF) signalling.

This study aimed to examine siponimod (2 mg/kg i.p.) impact on the alpha-synuclein (α-syn), glial markers of neuroinflammation, BDNF and another protein expressions crucial for neuronal function in rats SC after Th9 segment compression (40g/15min). Moreover, to better understand the mechanism of S1P receptor stimulation, the role of siponimod and another S1P receptor modulator – ponesimod in oxidative stress, evoked by pro-apoptotic C2-ceramide in human *neuroblastoma* SH-SY5Y cells was examined.

Western blot analysis indicated elevation of α-syn multimer weighting ~37 kDa and simultaneous reduction in ~55 kDa and higher aggregates in the SC epicentre of injury after siponimod administration. It's worth pointing out that α-syn is an intrinsically disordered protein, gaining toxic conformation/aggregation under stress conditions. Therefore, the reduction of larger α-syn multimers may be a rescue mechanism against SCI. Furthermore, a significant increase in Iba-1 and GFAP protein levels, specifically expressed by microglia and astrocytes, respectively, was reported in the SC epicentre of injury. Siponimod tended to diminish their up-regulation. No effect of siponimod on BDNF level in injured SC was observed. Still, PCR analysis indicated that this drug, like the other S1PR modulator–ponesimod, improved BDNF expression in SH-SY5Y cells, that was reduced by pro-oxidative C2-ceramide. Simultaneously, siponimod and ponesimod lessened BDNF receptor-neurotrophic tyrosine kinase receptor 2 (NTRK2) level, that was elevated by C2-ceramide, and surprisingly increased mRNA of α-syn and the ratio of pro/anti-apoptotic Bax/Bcl-2 proteins. Finally, both drugs protect cells against death/reduced metabolic activity, as measured by flow cytometry and MTT assay, respectively.

Current results suggest that siponimod ameliorates harmful uncontrolled inflammatory response and α-syn aggregation in rats' SC epicentre after traumatic injury. Moreover, Sphk1/S1P receptor signalling may offer protection by modulating BDNF-directed signalling.

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|A10|

TRP Gene Family: Important Mediators of Brain Development and Neurodegenerative Disorders

Olejniczak B.[1], Balakrishnan.[2], Augustyniak J.[3], Salinska E.[3], Godlewski J.[3], Bronisz A.[2]

[1]Department of NeuroOncology, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[2]Tumor Microenvironment Laboratory, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[3]Department of Neurochemistry, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Transient receptor potential (TRP) channels, as non-selective cation channels, play a pivotal role in maintaining cellular homeostasis and transmitting sensory signals. Their wide distribution across various tissues underscores their functional significance, particularly in the brain, where they regulate crucial processes such as pain sensation, temperature regulation, and pressure sensing. Recent studies have highlighted a significant role in brain development, particularly in the hippocampus, where they contribute to memory consolidation and neuronal function.

The study employed a comprehensive approach, delving into the distinct patterns of TRP channel expression using transcriptomic data from resources like the Human Protein Atlas and the Allen Brain Atlases. The focus was on developmental regulation, tissue-specific expression, aging-related changes, and neurodegenerative diseases like dementia. The findings revealed intriguing patterns of TRP channel expression, with genes like TRPM4, TRPC1, and MCOLN1 showing ubiquitous expression across all developmental stages, while TRPV2 and TRPM2 were predominantly expressed in postnatal stages. Additionally, TRPC3 displayed region-specific expression in the cerebellar cortex.

In aging and dementia, TRP channels were moderately deregulated, particularly TRPC5 in the hippocampus and TRPV1, TRPV5, and TRPM2 in white matter. Single-cell analyses revealed cell-specific shifts in TRP expression, with some channels, such as TRPV2 and TRPC7, being dementia-related, while others, including TRPM3 and TRPM7, remained unaffected by disease progression. Notably, oligodendrocytes displayed high-to-moderate TRP expression independent of dementia status, while GABAergic cortical interneurons exhibited dementia-dependent changes.

These findings are of paramount importance in understanding the role of TRP channels in both normal brain function and neurodegenerative disease progression. The spatial and temporal regulation of TRP gene expression offers profound insights into their biological significance and therapeutic potential. Particularly, in addressing sensory dysfunction and cognitive decline, these findings pave the way for new strategies for developing interventions for neurodegenerative disorders like dementia.

|A11|

Probe into neuroprotective effects of polyacrylic acid (PAA)-conjugated cerium oxide against MPP+-induced cell damage in SH-SY5Y cells

Meenambal R.[1,3], Jakubowska K.[1], Kruk T.[2], Warszynski P.[2], Danuta Jantas.[1]

[1]Maj Institute of Pharmacology, Polish Academy of Sciences, Smęta 12 Street, 31-343 Cracow, Poland
[2]Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Cracow, Poland
[3]Christ University, School of Psychological Sciences, Bangalore, India-560073

Cerium oxide nanoparticles have been widely explored against neurodegenerative diseases due to their antioxidant properties that aid in quenching reactive oxygen species. In our previous studies, we showed the neuroprotective effects of 0.03 M polyacrylic acid conjugated cerium oxide (PAA-CeO) against cell damage evoked by oxidative stress inducers, hydrogen peroxide and 6-hydroxydopamine in a cellular model of Parkinson's disease (human neuroblastoma SH-SY5Y cells) [1,2]. In order to extend a portfolio of PAA-CeO neuroprotective potency, we tested the effect of various concentrations of PAA-CeO (0.03 M, 0.05 M and 0.1 M) against cell damage evoked by MPP+ (1-methyl-4-Phenylpyridinium ion) in undifferentiated (UN-) and retinoic acid (RA)-differentiated SH-SY5Y cells. The size of synthesized nanoparticles varied in the range of 20-50 nm based on the concentration of PAA-CeO. The zeta potential of synthesized nanoparticles was between − 32 and − 35 mV. All tested concentrations of PAA-CeO were safe for UN- and RA-SHY5Y cells when given for 48 h. We found that dilutions 10-80x of 0.03 M PAA-CeO significantly attenuated the MPP+-evoked LDH release in UN- and RA-SH-SY5Y cells. However, higher concentrations of PAA-CeO (0.05 and 0.1 M) at any of the tested dilutions (10-40x) did not change the extent of cytotoxicity induced by MPP+. Moreover, we showed that neuroprotection mediated by 0.03M PAA-CeO against MPP+ is associated with the inhibition of apoptosis, as verified by caspase-3 activity and TUNEL labelling. The results point to the neuroprotective potential of PAA-CeO nanoparticles against neuronal cell damage induced by dopaminergic neurotoxin MPP+ in SH-SY5Y cells. This, combined with our previous findings from oxidative stress models [1,2], justifies the further investigation potential of these nanoparticles in animal models of Parkinson's disease.

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|A12|

Biomodeling of molecular mechanisms of repair of acrolein adduct to adenine and its clinical significance

Sokolowska B.[1], Dylewska M.[2], Poznanski .J[2], Maciejewska A[2].

[1]Bioinformatics Laboratory, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Acrolein is a ubiquitous environmental pollutant and endogenous metabolite that is beginning to be recognized as a serious threat to human health. People are exposed to acrolein through the respiratory/skin/oral tract. It has been linked to multiple sclerosis, Alzheimer's disease, cardiovascular disease or diabetes. At the cellular level, a variety of toxic effects of acrolein have been observed, including DNA and protein adduction, oxidative stress, mitochondrial dysfunction, membrane damage or endoplasmic reticulum stress. Its facile reactivity toward DNA can initiate mutagenesis, thereby contributing to the etiology of cancer. We present in vivo, in vitro and in silico evidence that acrolein adduct to adenine - 1,N6-α-hydroxypropanoadenine (HPA) is mutagenic and can be effectively repaired by E.coli adaptive response proteins - AlkA glycosylase and AlkB dioxygenase. To simulate endogenously arising adducts, we used acrolein-modified plasmids, allowing monitoring of all kinds of substitutions originating from acrolein modification of adenine. Both proteins were engaged in alleviating HPA-induced mutagenesis. Moreover, HPA was repaired by AlkA and AlkB in vivo, even without induction of adaptive response. HPA contains an asymmetric carbon atom in the hydroxypropane ring and exists as two stereoisomers. AlkA excises both of them in vitro. Kinetic data show, however, that AlkB preferentially repairs the protonated form of HPA, albeit the reaction is stereoselective. Molecular modeling of the T(HPA)T/AlkB complex showed that the R stereoisomer in the equatorial conformation of the HPA hydroxyl group is strongly preferred, while the S one seems to be susceptible to AlkB-directed oxidative hydroxylation only when HPA adopts the syn conformation around the glycosidic bond. In contrast, molecular modeling demonstrated how dsDNA carrying both HPA stereoisomers could be properly bound to the AlkA catalytic center. Thus, HPA repair by AlkA is not expected to be stereoselective. As non-homologues but acting similarly to AlkA, human 3-alkyladenine DNA glycosylase and human AlkB homologs involved in DNA repair are promiscuous enzymes that could also be engaged in HPA removal; these model studies provided insight into acrolein adducts repair mechanisms can be the basis for future prevention/treatment strategies in clinical practice.

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|A13|

Supporting (neuro)diagnosis and the effects (neuro)pharmacological therapy through novel (neuro)technologies in modern basic and clinical neuroscience

Sokolowska E.[1], Sokolowska B.[2], Sulejczak D.[3]

[1]Department of Developmental Psychology, Faculty of Social Sciences, Institute of Psychology, The John Paul II Catholic University of Lublin, Poland
[2]Bioinformatics Laboratory, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[3]Department of Experimental Pharmacology, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Modern information technology (IT), information and communication technology (ICT) and the Internet of Things (IoT) play an important role in basic/clinical neuroscience, especially in neuropsychology/neuropsychiatry/neurogeriatrics and neurology. An interesting aspect is the development of neurotechnology (with neurofeedback) to augment the therapeutic effects of traditional/non-pharmacological and/or pharmacological approaches in diseases of the nervous system, including natural ageing processes.

Our report presents promising results to date and future directions for the application of these technologies in neurodevelopmental/neuropsychiatric disorders, post-stroke, trauma or natural changes in the central nervous system (CNS). Algorithms/methods of Extended Reality (XR), Artificial Intelligence (AI) with robotics and Machine Learning (ML) also play an important role, as does the conceptual living/working of modern societies in the digital world, the so-called Metaverse.

Neurotechnologies and genetic tools for analyzing the function of neural circuits have advanced rapidly over the past decade. Understanding the precise pharmacological mechanisms of neuroactive compounds/drugs is crucial for the advancement of neuroscience and neuropharmacology, as well as for the development of more effective treatments for CNS disorders. As highlighted in the studies presented here, it is also important to integrate innovative tools to evaluate the effects of the neurointerventions undertaken.

Therefore, an equally important direction for assessing the impact of pharmacological treatment and (conventional and/or IT/ICT/XR) neurorehabilitation is the development of novel/digital psychometric strategies for analyzing the affective, cognitive and psychosocial functioning of these patient populations. The proposed modern/digital psychometric tools may be (a) even more sensitive than traditional approaches both in diagnosing/detecting symptoms/changes and (b) more precise in assessing the effects of new therapeutic interventions and modern rehabilitation programmes implemented, including XR/AI/ML.

In conclusion, the remarkable discoveries and promising results of ongoing interesting modelling studies and experimental/clinical interventions using state-of-the-art digital and computational (neuro)technologies, together with the IoT, are providing new and often more effective methods/approaches in both (neuro)diagnosis and (neuro)therapy, as well as supporting the (neuro)rehabilitation of patients with CNS dysfunction, injury and other serious disorders.

|A14|

Therapeutic potential of osthole in relation to differentiated and stem glioma cells

Sumorek-Wiadro J.[1], Scibiorski M.[1], Sklicka-Wozniak K.[2], Rola R.[3], Rzeski W.[1], Jakubowicz-Gil J.[1]

[1]Department of Functional Anatomy and Cytobiology, Institute of Biological Sciences,, Maria Curie-Sklodowska University, Lublin, Poland
[2]Independent Laboratory of Natural Products, Medical University of Lublin, Lublin, Poland
[3]Department of Neurosurgery and Pediatric Neurosurgery, Medical University of Lublin, Lublin, Poland

Gliomas are tumors of the central nervous system that have an extremely poor prognosis. One of the reasons is the presence of cancer stem cells (GSCs), which constitute a separate population of cells capable of intensive multiplication and differentiation. Unlike differentiated cells, GSCs are highly resistant to classical radio- and chemotherapy, and the inability to eliminate them is the main cause of disease relapse. Therefore, the use of compounds that combat not only differentiated tumor cells but also the population of cancer stem cells is so important in anti-glioma therapy.

Our research to date has shown that coumarins, in particular osthole, have enormous therapeutic potential in eliminating cancer cells of the central nervous system. Therefore, in this study, for the first time, assessed the anticancer properties of osthole in combination with temozolomide in relation to dedifferentiated and stem cells from primary glioblastoma cultures.

The studies used a primary glioblastoma multiforme culture and its stem cells, which were identified using flow cytometry based on the presence of CD133, CD44 and CD15 on their surface. Cells were incubated for 24 hours with osthole and temozolomide, in single and simultaneous application. A flow cytometry method was used to assess the level of apoptosis and necrosis. The obtained results were confirmed microscopically (Nikon-E800) on the basis of typical morphological changes, after prior staining of the cells with specific fluorochromes: Hoechst 33342 and propidium iodide.

Studies have shown that over 60% of stem cells contained the CD44 antigen, the presence of which indicates an extremely invasive – mesenchymal type of stem cells. It is characterized by a huge potential for self-renewal and high resistance to radiochemotherapy, so their presence is an unfavorable prognostic factor. As it results from our studies, the compound with high proapoptotic activity in relation to dedifferentiated and stem cells of glioblastoma multiforme is osthole. Coumarin eliminates approx. 60% of dedifferentiated cells and approx. 20% of stem cells by apoptosis. Interestingly, its combination with temozolomide is even more effective than a single application of osthole. This combination induces apoptosis in approximately 85% of dedifferentiated cells and 30% of stem cells.

|A15|

Inhibition of very long-chain fatty acids (VLCFAs) synthesis induces the formation of lipid droplets (LDs) in primary cell cultures of glioblastoma multiforme

Scibiorski M.[1], Sumorek-Wiadro J.[1], Hulas-Stasiak M.[1], Lange-Andrzejewska O.[2], Zwara A.[2], Mika A.[2], Sledzinski T.[3], Rola R, Jakubowicz-Gil J.

[1]Department of Functional Anatomy and Cytobiology, Institute of Biological Sciences, Maria Curie-Skłodowska University, Lublin, Poland
[2]Department of Environmental Analysis, Faculty of Chemistry, University of Gdansk, Gdansk, Poland
[3]Department of Pharmaceutical Biochemistry, Faculty of Pharmacy, Medical University of Gdansk, Poland
[4]Department of Neurosurgery and Paediatric Neurosurgery, Faculty of Medicine, Medical University of Lublin, Lublin, Poland

Glioblastoma multiforme (GBM) are the most malignant and common tumours of Central Nervous System. GBM cells are characterised by its high invasiveness which is the consequence of their ability to diffuse spread among normal cells. Current therapeutic strategies involves surgical resection of the tumour, radio- and chemotherapy coupled with temozolomide (TMZ). These actions are intended to prolong and improve the quality of patient's life which does not exceed 16 months. Due to the high resistance of GBM cells to current therapies, molecules that better penetrate cell membranes or increase their permeability allowing more efficient entry of anticancer compounds have become a main target.

Our studies to date performed by gas chromatography coupled with mass spectrometry (GC-MS) have shown that primary cell cultures of GBM are characterised by an increased levels of very long-chain fatty acids (VLCFAs) synthesised by ELOVL1 elongase (very long chain fatty acid elongase 1), which could be responsible for the resistance of these cells to induction of programmed cell death and high migration potential. Due to metabolic reprogramming of cancer cells, which is able to increased lipid uptake and synthesis, important role in their maintenance may play lipid droplets (LDs), which are reservoirs of neutral lipids and involved in maintain cellular energy balance, lipid homeostasis and signaling

Therefore, the aim of our study was to investigate the effect of the inhibition of VLCFAs synthesis by specific inhibitor of ELOVL1 elongase on lipid droplets formation in GBM primary cell cultures. The viability of GBM cells treated with ELOVL1 inhibitor was determined by MTT Assay. Oil Red O dye and hematoxylin was used to visualise lipid droplets and nucleus, respectively. The level of VLCFAs after treatment with ELOVL1 elongase inhibitor was analysed gas chromatography coupled with mass spectrometry.

The results have shown that the decreased level of VLCFAs in GBM primary cell cultures was characterised by the increased presence of lipid droplets. This results suggest that lipid droplets may be a reservoirs of fatty acids which have less than 22 carbon atoms in the structure.

|A16|

Integrative model of GSCs transcriptome responding to ferroptosis induction and oxygen availability

Tunçarslan S.[1], Balakrishnan A.[1], Ciecwierz M[1], Lewandowska-Gnatowska E.[1], Godlewski J.[2], Bronisz A.[3]

[1]Tumor Microenvironment Laboratory,, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[2]Department of NeuroOncology, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[3]Tumor Microenvironment Laboratory, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Glioblastoma, a highly aggressive brain tumor, is characterized by glioblastoma stem-like cells (GSCs) that are resistant to conventional apoptosis-inducing therapies. These cells adapt to and survive within hostile tumor microenvironments, making treatment difficult and rendering traditional approaches inadequate due to their robust defense mechanisms. This challenge necessitates the development of innovative therapeutic strategies.

This study presents an integrative approach to targeting GSCs by inducing ferroptosis, a form of cell death to which these cells have not exhibited resistance. Using next-generation sequencing, we analyzed the transcriptional responses of GSCs treated with a ferroptosis inducer under both normoxic and hypoxic conditions, which reflect the varying microenvironmental challenges these cells face. The project leverages the FerrDb V2 database for ferroptosis gene signatures and incorporates comparative gene expression analysis using the TCGA and Ivy GAP databases. This approach enables a comprehensive examination of ferroptosis gene expression across volatile tumor microenvironments and normal tissues, thereby improving our understanding of GSC behavior across diverse pathophysiological conditions.

To support broader research and analysis, we developed 'The GSCsAtlas', a platform that integrates these datasets with robust visualization tools. The platform is compatible with all web browsers and does not require extensive computing resources, providing a user-friendly interface for researchers to explore GSC heterogeneity and plasticity in depth. The code has been deposited on GitHub, and the data are available from the internal IMDiK server at http://10.0.8.210:8080/.

Our research shows that triggering ferroptosis in GSCs is promising as a therapeutic approach, particularly regarding the amount of oxygen in the environment. When GSCs were treated with RSL3, a ferroptosis inducer, gene expression profiles changed according to the available oxygen, and this treatment was associated with improved patient survival. The survival benefit was linked to changes in immune response genes rather than ferroptosis-related ones. Our analysis also revealed that the genes most significantly increased in response to RSL3 were ferroptosis suppressors, indicating that GSCs reprogram themselves to bypass cell death pathways. These results suggest that combining ferroptosis with immunotherapy would benefit more than relying only on cell death-inducing strategies.

|A17|

The role of Bcl-2:beclin-1 complex in sensitizing the glioma cells to apoptosis induction upon inhibition of pathways regulated by TrkB receptor

Zajac A., Sumorek-Wiadro J., Scibiorski M., Rzeski W., Jakubowicz-Gil J.

Functional Anatomy and Cytobiology, Institute of Biological Sciences, Maria Curie-Sklodowska University, Lublin, Poland

The most aggressive tumors of the human central nervous system are anaplastic astrocytoma (AA, III grade) and glioblastoma multiforme (GBM, IV grade) with an extremely bad prognosis. Their malignant character and resistance to standard therapy are associated with the over-expression of intracellular survival pathways such as Ras/Raf/MEK/ERK and PLCγ1/PKC regulated by TrkB receptor. It is also correlated with apoptosis avoiding and autophagy promoting. In our previous study we observed that the Blc-2:beclin-1 complex acts as a specific molecular "switch" and its formation lead to apoptosis promotion and autophagy decrease.

Therefore, the aim of this study was to investigate the engagement of those pathways in human glioma cells resistance for apoptosis induction by Temozolomide treatment and what is the role of Bcl-2:beclin-1 complex formation in this mechanism.

Two cancer MOGGCCM (AA) and T98G (GBM) and one normal human astrocytes (NHA) cell lines were used for analysis. The TrkB (LOXO-101), Raf (Sorafenib) and PLCγ1 (U-73122) inhibitors in single and simultaneous action with Temozolomide on apoptosis induction was analyzed by microscopic observations of characteristic morphological changes of the cells. Bcl-2:beclin-1 complexes occurrence was also assessed by immunofluorescence assay. For blocking TrkB, Raf and PLCγ1 gene expression specific siRNA was used.

Tested drugs effectively eliminate cancer cells, apoptosis was the dominative type of death what was accompanied with Bcl-2:beclin-1 complex formation. Inhibiting the pathways regulated by TrkB receptor combined with Temozolomide action, led to successful gliomas elimination.

The received results extended the knowledge about the role of intracellular survival pathways regulated by TrkB receptor in glioma cells drug resistance and the engagement of the specific molecular "switch" – Bcl-2:beclin-1 complex in their sensitizing to apoptosis induction, and might serve as the basis for modern targeted treatment development.