Session 8: Signaling pathways and cellular regulation

Lectures

L.08.1

The nuclear factor kappa B (NFκB) signaling pathways in preeclamptic placental cells

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Preeclampsia (PE) is disorder occurring in pregnancy, generally after week 20 of gestation. It is recognised as a sudden onset of hypertension, i.e. >140/90 mmHg in previously normotensive women, Although it is commonly complicated by proteinuria, it can be accompanied by other comorbidities such as renal or liver dysfunction, haematological or neurological disorders, or placental insufficiency. Although PE has long been recognised, its precise pathomechanism remains poorly understood. It is known, that the source of PE appears at the beginning of gestation i.e. several weeks before the manifestation of the first clinical symptoms and it is strongly related to the inflammation. Both maternal blood and preeclamptic placentas demonstrate high concentrations of inflammatory markers e.g. interleukins 1, 6 or 8 and tumour necrosis factor type alpha (TNFα), as well as various anti-angiogenic or pro-apoptotic factors. Most of the genes coding for these factors are controlled by the transcription factor kappa B (NFκB), whose elevated level and activity are characteristic of preeclampsia. Unfortunately, the mechanism of NFκB activation, especially in placental tissue, also remains unclear. The evidence suggests NFκB is not activated by the three best-known signaling pathways: viz. classical, non-classical and alternative. Therefore, this presentation will discuss the most probable preeclamptic mechanism of NFκB activation in placental cells, and its consequences.

L.08.2

Synaptic deficiency in the iPSC model of Alzheimer's disease

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A model of neurons and brain organoids derived from induced pluripotent stem cells (iPSCs) offers a groundbreaking way to study the neurodegeneration of Alzheimer's disease (AD). These three-dimensional brain organoids reflect the development and organization of the human brain. Using iPSC technology and miniaturized brain-like structures, we can unravel the complex cellular and molecular causes of AD-related synaptic deficits. Differentiation of iPSCs in cerebral organoids yields appropriate neural networks, revealing synaptic aberrations reminiscent of the brains of AD patients. Molecular studies of such brain organoids advance our understanding of disrupted synaptic proteins, neurotransmitters and calcium signaling, affecting synaptic communication in AD. Our team investigates, among others: role of pentraxins in synaptic deficiency in this AD brain model. In addition, the iPSC brain organoid model facilitates drug discovery for AD therapy by screening compounds that correct synaptic deficits. Overall, iPSC-derived brain organoids are pioneering the study of AD-related synaptic issues, offering insight into molecular causes and screening for potential treatments.

L.08.3

Dysregulation of adrenergic excitability in astrocytes in neurodegeneration

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Astrocytes, an abundant and functionally heterogeneous neuroglial cells, are essential for maintaining brain homeostasis. Astrocytes are rich in adrenergic receptors and are considered the main effectors of the noradrenergic system. During attention, wakefulness, and stress, the noradrenergic system of the nucleus *locus coeruleus* activates the brain by releasing noradrenalin. This stimulates brain metabolism, which is important for memory formation and learning. Activation of astroglial adrenergic receptors leads to an increase in intracellular Ca^{2+} and cAMP, activating aerobic glycolysis with L-lactate production. L-lactate is an important energy fuel transported from astrocytes to neurons to support neuronal functions, including cognition. Impairment of the noradrenergic system is associated with neurodegeneration-related cognitive decline. How this affects astrocyte function and neuronal support is unclear. We have recently demonstrated impaired adrenergic signalling, lipid and glucose metabolism, and L-lactate release in astrocytes that form cytoplasmic TAR DNA-binding protein 43 (TDP-43) inclusions, a hallmark of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). This suggests that adrenergic activation of astrocytes and their ability to homeostatically support neurons are impaired, which may contribute to neurotoxicity, neurodegeneration and cognitive decline. Thus, astrocytes may represent a new target for the treatment of neurological diseases.

L.08.4

Alpha-synuclein: a surprising activator of Ca^{2+}-transporting ATPases

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Alpha-synuclein (aSN) is a membrane-associated, intrinsically disordered protein known for its pathological aggregation in neurodegeneration. The role of aSN in physiological functions, however, remains debated. The Plasma Membrane Calcium ATPase (PMCA) is an enzyme essential for cellular calcium homeostasis, regulated by mechanisms like alternative splicing, acidic phospholipids, and interactions with calcium-bound calmodulin (Ca^{2+}-CaM). Our findings reveal that the aSN strongly activates PMCA when paired with negatively charged phospholipids. Soluble, monomeric aSN influences turnover rate and calcium affinity, independent of PMCA's autoinhibitory/CaM-binding domain. Instead, activation hinges on the lipid environment and the anchoring N-terminus of aSN. Mutagenesis studies show aSN binds PMCA's lipid-sensory site located in the in the A-domain-TM3 linker. Transcriptomics data suggest aSN-rich tissues favor less Ca^{2+}-CaM-dependent PMCA splice variants. aSN accumulating in neuronal presynaptic termini, likely complements calmodulin. Additionally, to grasp the implications of lipid-driven aSN binding to PMCA and to evaluate its impact on calcium levels in the presynaptic terminal cytosol, we formulated a mathematical model for regulating calcium ions in the presynaptic environment.

Oral presentations

O.08.1

MCPIP1 inhibits hepatic stellate cell activation in autocrine and paracrine manner

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Background and Aims: During hepatic fibrosis stellate cells (HSCs) activate and secrete extracellular matrix proteins, forming a scar tissue, which results in liver dysfunction. In this study, we analyzed MCPIP1 level in human fibrotic livers and hepatic cells isolated from murine fibrotic livers. We also investigated MCPIP1 impact on HSCs activation.
Methods: We analyzed MCPIP1 level in patients' fibrotic livers and hepatic cells isolated from fibrotic murine livers and LX-2 cells line. Paracrine effect of Mcpip1 on HSCs activation was studied by coculture of Mcpip1 KO or WT primary hepatocytes with HSCs.
Results: MCPIP1 level is induced in patients' fibrotic livers in comparison to non-fibrotic counterparts. Similarly, both mRNA and protein Mcpipi1 levels were induced in primary HSCs isolated from murine fibrotic livers in comparison to control cells. Mcpip KO hepatocytes were characterized by increased expression of Ctgf protein than control cells, that resulted in enhanced activation of cocultured HSCs. Overexpression of MCPIP1 in LX-2 cells led to decreased mRNA expression of HSCs activation markers e.g. Acta2, Tgfb, Col1a1 and α-SMA protein level. Contrary, MCPIP1 silencing in LX-2 cells resulted in their increased activation status. Our results indicate that MCPIP1 could have a potential role in development or resolution of liver fibrosis.

Acknowledgments

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O.08.3

Role of cellular senescence and autophagy in colon cancer cell chemoresistance and stemness phenotype: role of hypoxia

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Colon cancer treatment using chemotherapy shows limited therapeutic efficiency due to chemoresistance. Autophagy and therapy-induced senescence (TIS) are purported chemoresistance mechanisms, with TIS believed to induce phenotype changes also present in cancer stem cells.
In vitro experiments were performed using two cell culture variants: normoxia (21% O_2) and hypoxia (1% O_2) to determine if autophagy inhibition coupled with oxygen deprivation has a senolytic effect or hinders the ability of senescent cells to divide/produce progeny. Main phenotype-test experiments were performed on HCT116 cell line treated with irinotecan (IRINO) to induce senescence, and hydroxychloroquine (HCQ) to inhibit autophagy.
HCT116 cell line exhibited traits of senescence in normoxia and hypoxia after treatment by 5 μM IRINO (24 h incubation), based on the increased fraction of polyploid and G2/M phase-arrested cells as well as SA-β-gal positive cells; however, senescence escape was recorded five days post-incubation. Additionally, in hypoxia, a decrease of p21 and E-cadherin levels was observed, suggesting a role of hypoxia in senescence escape. HCQ did not significantly influence the senescence markers expression, however the p62 and LC3BII/LC3BI shifts show that HCQ induces additional stimulation of autophagic flux rather than its inhibition, in the present *in vitro* model.

Acknowledgments

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Posters

P.08.1

Verification of the senescence algorithm in patients with lung cancer treated with chemotherapy

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Recurrence after chemical treatment is one of the problems of modern oncology. Cellular senescence, and especially the escape from cellular senescence associated with atypical divisions of senescent/polyploid cells, may be one of the potential mechanisms of chemo-resistance. In our ongoing *in vitro* and *in vivo* cancer studies in mice, we have followed the expression of senescent cell markers and of escape from senescence. The present study aimed to verify their algorithm on samples from lung cancer patients.
The research was carried on fragments of tumors and blood taken from chemotherapy-treated and untreated patients with lung cancer. Samples were analyzed using q-PCR, immunohistochemical staining, and ELISA. We performed an analysis of the expression of gene/protein related to senescence, proliferation, stemness, hypoxia, and epithelial to mesenchymal transition. Then, molecular data have been correlated with clinical data: TNM Classification of Malignant Tumors (Tumor Node Metastasis), the histological grade of cancer tissue differentiation, and the type and results of the test for the presence of eventual gene mutations.
Our preliminary data suggest that senescence may contribute to reduced patient resilience to cancer therapies and may provide a pathway for disease recurrence after cancer therapy. We believe that our research will allow us to develop algorithms that will support the process of diagnosing oncological patients, and monitor the effects of treatment.

P.08.2

Vismodegib and the Wnt canonical pathway inhibitor PRI-724 affect tongue squamous cell carcinoma CAL 27 cell line

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The dysregulation of signaling pathways is important for the development of head and neck squamous cell carcinoma (HNSCC). We aimed to evaluate the effects of individual and combined inhibition of Hedgehog (Hh) and Wnt pathways in tongue cancer cells.
Vismodegib (Hh pathway inhibitor), PRI-724 (Wnt/β-catenin pathway inhibitor), and CAL 27 cell line were used. The resazurin assay was used to determine IC25 and IC50 values. Cell cycle distribution and apoptosis were detected by flow cytometry after staining with propidium iodide or Annexin V, respectively. The scratch assay was performed to analyze cell migration and real-time PCR was done to evaluate the expression of genes associated with cancer stem cells (CSC).
The IC25 and IC50 values were 2.6 μM and 8.3 μM for PRI-724, and 18.0 μM and 39.0 μM for vismodegib, respectively. Vismodegib (IC25, IC50) and PRI-724 (IC25) induced cell cycle arrest in G1/G0 phases, but PRI-724 (IC50) mixed with vismodegib (IC25) worked in the opposite direction. Apoptosis was induced in all experimental cases except for the lower concentration of PRI-724 and its mixture with vismodegib (IC50). The combination of PRI-724 (IC50) and vismodegib (IC25) synergistically reduced cell migration by 80%. Moreover, vismodegib effectively down-regulated the expression of *ALDH1A1*, *SOX2*, and *POU5F1*.
The inhibitors of Wnt and Hh pathways can attenuate the migration of tongue cancer cells and reduce the expression of CSC markers.

P.08.3

The signaling role of ergothioneine

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Ergothioneine is a naturally synthesized metabolite produced by fungi, yeast and bacteria. Due to its high accumulation, low excretion, and ubiquitous presence in the human body, ergothioneine has been called the longevity vitamin. Since the discovery of its transporter in 2005, ergothioneine is experiencing its second youth. Ubiquitously expressed SLC22A4 imports ergothioneine to cells and is present even in the blood-brain barrier. Available data suggests that ergothioneine has beneficial effect on neurons and can e.g. revert depression-like behavior in mice. Most of the ergothioneine properties are usually associated with its antioxidative activity, while some of them may be an effect of its signaling role. However, there is still little data about its potential role as a signaling molecule.
Here, we present data supporting the hypothesis that ergothioneine functions not only as an antioxidant, but also exerts specific signaling based on the type of cells. Pretreatment of HepG2 cells with ergothioneine modulated the activity of some of the ABC-transporter family members. Additionally, ergothioneine changed the cellular response to treatment with lipopolysaccharide and some of the cytostatic drugs. Meanwhile, glioblastoma SH-SY5Y cells were more resistant to high concentrations of ergothioneine. Additionally, the analysis of protein level revealed, that GSK-3β pathway may be involved in the mechanism of action of ergothioneine.

P.08.4

The Role of Axin1 in the insulin-dependent regulation of glucose uptake in cultured rat podocytes

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Insulin affects the morphology and function of podocytes, which regulate the permeability of the glomerular filtration barrier. Disturbances in insulin signaling impair glucose uptake, which may lead to the development of insulin resistance in podocytes, and in consequence, alter glomerular filtration. Axin1 is a scaffold protein linked to the regulation of glucose uptake in cells. Therefore, the Axin1 involvement in controlling insulin-dependent glucose uptake in cultured rat podocytes was studied.
To determine the impact of Axin1 on the insulin-dependent phosphorylation of insulin receptor (IR) and its membrane localization, the expression of gene coding Axin1 was knocked down by small interfering RNA (siRNA). Glucose uptake was measured using (1,2-^{3}H)-deoxy-D-glucose.
Axin1 siRNA decreased phosphorylation of IR in the presence of insulin by 43% (0.448 ± 0.053 *vs.* 0.259 ± 0.029, $n=5$-6, $P<0.01$ compared to insulin alone) without affecting the cell surface localization of IR. Downregulation of Axin1 expression by siRNA inhibited glucose uptake in response to insulin (1.169 ± 0.055 *vs.* 0.966 ± 0.052, $n=14$, $P<0.05$ compared to insulin alone).
These results suggest that Axin1 is involved in the insulin-dependent regulation of glucose uptake in cultured rat podocytes. Altered Axin1 activity may impair insulin signaling to downstream effectors, leading to insulin resistance in podocytes.

Acknowledgments

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P.08.5

GPR81 regulates podocyte lipid accumulation in hyperglycemia

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Podocytes are the crucial part of glomerular filtration barrier and their foot processes effacement is considered the primary cause of proteinuria in diabetic nephropathy. Over the years lipotoxicity among hyperglycaemia (HG) has been associated with renal damage. Altered lipolysis seems to be a part of lipotoxicity origin. It has been acknowledged that lactate responsive G-protein-coupled receptor (GPR81) activation is associated with lipolysis inhibition. We hypothesize that lactate upregulation in podocytes in HG is responsible for lipolysis suppression through GPR81 receptor signalling. The expression of both mRNAs and proteins in podocytes/glomerulus were determined using qPCR, western blot under HG (30 mM, 5 days). We used BODIPY 493/503 to detect the lipid droplets in podocytes. Glycerol amount (ELISA kit) was measured under GPR81 altered expression (GPR81 mRNA silencing with siRNA transfection). Our data reveals downregulation of GPR81 mRNA/protein expression in podocytes in HG and in diabetic rat glomerulus. Accumulation of lipid droplets in podocytes during lactate incubation, HG and GPR81 siRNA was observed. HG downregulated both the Perilipin and ATGL expression. Glycerol amount was decreased in GPR81 siRNA. Our results suggest that hyperglycemic-dependent downregulation of GPR81 impairs lipid metabolism and it might contribute to the development of diabetic nephropathy.

Acknowledgments

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P.08.6

Interaction of multivalent galectins with N-glycans attached to fibroblast growth factors fine-tuning their cellular signaling

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The human fibroblast growth factors (FGFs) are a family that comprises 22 proteins that employ their receptors (FGFRs) and regulate crucial cellular processes such as cell division, metabolism, differentiation, migration and apoptosis. The vast majority of FGFs is released by cells using a classical secretory pathway where their N-glycosylation may occur. However, the functional significance of these modifications remains largely unknown. We have recently identified a specific set of extracellular lectins, galectins −1, -3, -7 and −8, as novel interacting partners, specifically binding N-glycans of FGFs. We demonstrate that distinct galectins differentially modulate FGF4 signaling and FGF4-dependent cellular processes. Moreover, galectins multivalency is strictly required for the binding of FGF4 N-attached glycans and capturing of FGF in the extracellular matrix, creating a reservoir of the growth factor in the proximity of FGFRs. Our data reveal a novel regulatory module of FGF/FGFR signaling regulation, in which the information stored in N-glycans of FGFs is differentially deciphered by multivalent galectins and used to fine-tune cellular signaling.

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P.08.7

Receptor clustering mechanism is employed by galectins to control transduction of signals by FGFRs

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Fibroblast growth factor receptors (FGFRs) and their canonical ligands, fibroblast growth factors (FGFs) form signaling platforms at the cell surface which are critical for the regulation of cellular processes, such as cell division, motility, metabolism and death. FGFRs are N-glycosylated at several positions, however the significance of these modifications is still mysterious. Galectins are carbohydrate-binding proteins implicated in immune response, inflammation, cell division, motility and death. We demonstrated that a precise set of extracellular multivalent lectins, galectins -1, -3, -7 and -8, bind N-glycans in D3 domain of FGFRs and adjust FGFR signaling and cell physiology at multiple levels. Using engineered galectins with different valency, we showed that N-glycosylation-dependent clustering of FGFR1 constitutes a mechanism for FGFR1 stimulation by galectins. Importantly, the transmission of signals by galectin/FGFR complexes has different consequences for the cell physiology, affecting on cell viability and metabolic activity. Summarizing, our data identify a novel regulatory module in FGFR signaling, where N-glycosylation of the receptor provides the information, that distinct members of the galectin family differentially regulate fine-tune FGF/FGFR signaling and determine cell performance.

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P.08.8

Differences in response of 2D and 3D breast cancer cultures against modulation of estrogen-dependent signal pathways

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Estrogen-dependent signalling is crucial for homeostasis of the mammary gland epithelium. Studying the mechanism of estrogen-dependent signalling and its' disruption via estrogen mimetics is still a relevant issue, especially for breast cancer etiology. 2D cell cultures does not mimic a variety of interactions between cells, which is present in vivo and does not simulate changes in the tumour microenvironment due to its metabolic activity. Hence, the optimisation of 3D cell cultures is an important problem in basic research focused on breast cancer biology.

Our work aimed to compare the response of 3D and 2D cell cultures of MCF-7 cells against supplementation with estrogen and/or cytostatic drugs like Tamoxifen. 3D cultures were prepared using the Hanging Drop Method and the supplementation of medium with methylcellulose. We have measured the viability (I) and proliferation (II) of both types of cultures after incubation with the drug. Moreover, screening of changes in estrogen-dependent gene transcription was performed.

Results have shown, that 3D cultures exhibit different responses against tamoxifen (enhanced resistance to a toxic concentration of the drug and induction of proliferation). Moreover, 3D and 2D cell cultures exhibited different profiles of estrogen-dependent gene expression (both up and down-regulation). In summary, our work shows that 2D and 3D breast cell cultures exhibit a different both phenotype and response to the estrogen signalling.

P.08.9

The role of N-glycosylation in FGFR1 trafficking between the plasma membrane and the nuclear envelope

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Fibroblast growth factors (FGFs) together with fibroblast growth factor receptors (FGFRs) constitute a signaling system controlling fundamental cellular processes. The abnormal FGFR1 is frequently observed in various cancers. FGFR1 is a heavily N-glycosylated receptor tyrosine kinase, which apart from the plasma membrane, was also found in the nuclear lumen. However, the exact mechanism of FGFR1 nuclear transport is still unknown.
Here we constructed a glycosylation-free (GF) mutant of FGFR1, FGFR1.GF, and showed its primary localization to the nuclear envelope. We demonstrated that inefficient secretion is responsible for FGFR1 accumulation in the nuclear envelope. We also generated N-glycosylation mutants of FGFR1 and demonstrated that only the simultaneous presence of N-glycans of the immunoglobulin-like domains D2 and D3 of FGFR1 enables the efficient transport of FGFR1 to the plasma membrane. In addition, we observed that FGFR1.GF displays a high level of autoactivation, which suggests the presence of nuclear FGF-independent signalling. Using mass spectrometry and proximity ligation assay we identified novel binding partners of the FGFR1.GF. These data indicate that N-glycosylation of FGFR1 has an important role in the regulation of FGFR1 kinase activity and trafficking between the nuclear envelope and plasma membrane, which impacts the cellular function of the receptor.

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P.08.10

Does physical effort cause changes in expression of selected chemokine and interleukin receptor genes in peripheral blood leukocytes?

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It is postulated that lifestyle can induce unique life-associated molecular patterns (LAMPs). The study aimed to assess the post-effort transcriptional changes in selected genes encoding receptors for chemokines and interleukins in young, physically active men to better understand the immunomodulatory effect of physical activity. The participants aged 16-21 years, performed physical exercise tasks of either a maximal multistage 20 m shuttle-run test or a repeated speed ability test. The expression of selected genes encoding receptors for chemokines and interleukins in nucleated peripheral blood cells was determined using RT-qPCR. Aerobic endurance activity was a positive stimulant that induced increased expression of *CCR1* and *CCR2* genes following lactate recovery, while the maximum expression of *CCR5* was found immediately post-effort. The increase in the expression of inflammation-related genes encoding chemokine receptors triggered by aerobic effort strengthens the theory that physical effort induces sterile inflammation. Different profiles of studied chemokine receptor gene expression induced by short-term anaerobic effort suggest that not all types of physical effort activate the same immunological pathways. A significant increase in *IL17RA* gene expression after the beep test confirmed the hypothesis that cells expressing this receptor, including Th17 lymphocyte subsets, can be involved in the creation of an immune response after endurance efforts.

P.08.11

The role of EGFR redistribution in the electrotaxis of mouse 3T3 fibroblasts

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Electrotaxis, the directed migration of cells in an electric field (EF), is crucial for wound healing and embryonic development. While the precise mechanism of the EF detection remains unclear, it is believed that the redistribution of membrane proteins, such as chemoattractant receptors, driven by the EF, plays a key role in cell polarization and directional migration.
This study regarded the role of the EGF receptor (EGFR) in electrotaxis of mouse 3T3 fibroblasts. We confirmed the cathodal electrotaxis of cells and its EF strength dependence. Inhibiting EGFR partially disrupted electrotaxis. Then we visualized the localization of EGFR-GFP before and after EF exposure. As expected, EGFR accumulated on the cathodal side of the cell. The dynamics of redistribution was pH dependent, with the highest efficiency in the alkaline conditions, while the final fluorescence distribution was dependent on the EF intensity. The redistribution of EGFR correlated well with the response of the cell to EF application, but not with the rapid response of 3T3 fibroblasts to EF reversal, which was visible in 1-2 min, i.e. when the previous receptor polarity was still maintained.
In summary, it was shown, that the mechanism of EF detection with EGFR redistribution (possibly via electroosmosis) is involved in the electrotaxis of 3T3 fibroblasts, but cannot explain so quick response to EF reversal.

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P.08.12

Ciliary gene *TRIP11* suppresses adhesion and migration of renal cancer cells

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Background:. Primary cilia present on epithelial cells that line the renal tubules are involved in signal detection and transduction. Recently we found downregulation of 26 cilia-associated genes in clear cell renal cell carcinoma (ccRCC), including TRIP11 which emerged as an inhibitor of cancerous proliferation and viability, and a target of miR-155-5p that affected cilium length. Here, we aimed to extend our study on the functional significance of TRIP11 in ccRCC. **Material and method:** TRIP11 expressing plasmid or empty plasmid (pJAF205) was transfected in Caki-1 and 786-O cell lines. Silencing of TRIP11 was induced using siRNA. TRIP11 overexpression/silencing was confirmed using Immunocytochemistry and Western blot. Adhesion, migration, and invasion assays were performed to investigate TRIP11 involvement in cancer. **Results:** TRIP11 overexpression in metastatic renal cancer cells inhibited the adhesion and migration of ccRCC cells. **Conclusions:** Our current and previous data together indicate that loss of the TRIP11 cilium regulatory gene in renal cancer may lead to enhanced cancerous proliferation, migration and adhesion.

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P.08.13

BacSp222 as the first proinflammatory bacteriocin recognized by TLR2/TLR6 heterodimer

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BacSp222 bacteriocin is a 50-amino acid-long peptide produced by a zoonotic *Staphylococcus pseudintermedius* 222 strain. This molecule is the first known factor that combines the features of bactericidal peptide, virulence factor, and proinflammatory molecule (Wladyka B. et al., 2015, Sci. Rep. 5, 14569). It kills Gram-positive bacteria, has cytotoxic activities against eukaryotic cells and stimulates immune cells to produce selected cytokines, such as TNF or IL-8, in NF-κB-dependent manner (Śmiałek J. et al., 2022, J. Inflamm. Res. 15, 4601-4621). Recently it was demonstrated that BacSp222 stimulated NF-κB activation in HEK-Blue cells overexpressing human toll-like receptors 2 (TLR2) and, simultaneously, this phenomenon has not been observed in cells overexpressing TLR4 or TLR5. Further studies have shown that BacSp222 is specifically recognized by the TLR2/TLR6 heterodimer. Stimulation of the TLR2/TLR6 heterodimer by BacSp222 was inhibited with the TLR2-specific antagonists, that reduced activation of NF-κB signaling in BacSp222-stimulated HEK-Blue TLR2/TLR6 cells, as well as reduced TNF release by BacSp222-treated RAW 264.7 and P388.D1 cells (Śmiałek-Bartyzel J. et al., 2023, Inflamm. Res. 72, 915-928). The presented results are the first report demonstrating that the eukaryotic receptor from the pattern recognition receptors (PPR) family can be responsible for recognizing bacteriocin molecule.

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P.08.14

The biochemical characterization of Dicer1e, a truncated variant of human ribonuclease Dicer

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Dicer ribonucleases play a pivotal role in the biogenesis of small regulatory RNAs by processing long double-stranded RNAs (dsRNAs) and single-stranded hairpin RNA precursors (pre-miRNAs) into small interfering RNAs and microRNAs (miRNAs), respectively. Human Dicer (hDicer) is a multidomain enzyme comprising a putative helicase domain, a DUF283 domain, platform, a PAZ domain, a connector helix, two RNase III domains (RNase IIIa and RNase IIIb) and a dsRNA-binding domain (dsRBD). In all vertebrates, only one gene encoding the Dicer protein has been identified. The hDicer-coding gene is named: *DICER1*. From *DICER1* gene, multiple Dicer transcript variants can be produced as a result of the initiation of transcription from alternative promoters and alternative splicing. Our studies focus on the variant named Dicer1e, that is generated through alternative splicing. The Dicer1e protein product has a unique N-terminus, and comprises only the two RNase III domains and dsRBD. Dicer1e is expressed both in healthy and tumor cells. Thus far, the biochemical activity of the Dicer1e protein has not been investigated. Using the in *vitro* cleavage assay, we demonstrated that the Dicer1e protein cannot cleave pre-miRNAs and dsRNAs. Instead, it could hydrolyze miRNA-size substrates, suggesting that this variant can be involved in RNA turnover in the cell. These results were also supported by the experiments carried out on human cells overexpressing the Dicer1e variant.

P.08.15

Impact of nutrient availability and hypoxia-inducible factors on breast cancer invasive potential

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Metastases cause most deaths related to breast cancer, so it is important to understand better the molecular mechanisms underlying their occurrence. Despite the name, hypoxia-inducible factors (HIFs) transcriptional activity is not limited to insufficient oxygenation. Their activity is believed to enhance malignant features, including invasive potential. Most studies concerning the HIFs are focused on the HIF1 isoform and are performed on cells grown in excess of glucose and glutamine. The study aimed to investigate various conditions and distinguish between the roles of HIF1 and HIF2 isoforms and how they affect the invasive potential of breast cancer, as nutrient availability might present an essential regulator of hypoxia-inducible factors. The analysis of invasion and migration was based on different breast cancer cell lines cultured in various nutrient availability. Because co-occurring metabolic diseases might result in developing a more aggressive phenotype of breast cancer, the study included an analysis of clinical samples. Samples were analyzed regarding HIF and HIF2 isoform levels and their correlation with characteristics such as metastases and diabetes occurrence. The results showed different tendencies between HIF1 and HIF2 isoforms concerning divergent nutrient availability and metastases statuses, as well as distinct effects of both isoforms on the invasive potential of breast cancer.

P.08.16

Rb1 protein is not responsible for induction of *TGF-β2* gene expression after $BRAF^{V600E}$ kinase inhibition with PLX4720

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The proto-oncogene serine-threonine kinase BRAF plays an important role in cell proliferation, differentiation, and maturation by activating the MAPK (mitogen-activated protein kinase) signaling pathway. A missense mutation in *Braf* gene ($Braf^{V600E}$) is present in several human cancers such as melanoma, lung cancer and liver cancer. Vemurafenib, is a $BRAF^{V600E}$ kinase-targeting drug used as a treatment strategy for melanoma. However, most patients treated with Vemurafenib develop resistance due to not fully elucidated mechanisms. We found that PLX4720 (an inhibitor of $BRAF^{V600E}$) treatment increased expression of *TGF-β2* gene in melanoma and liver cancer cell lines.
To elucidate the mechanisms, we hypothesized that Retinoblastoma 1 (Rb1) tumor suppressor protein, which is inactivated by MAPK and has been implicated in inducing *TGF-β2* gene expression, may be at play. We found, that Rb1 protein levels were reduced, whereas mRNA levels were unchanged after $BRAF^{V600E}$ activity with PLX4720. As expected, phosphoERK levels were also reduced. Similar effect of reduced Rb1 protein level was observed after blocking Cyclin-dependent Kinases (which are downstream of MAPK and phosphorylate Rb1). Moreover, in cells with a stable knockdown of *Rb1*, *TGF-β2* expression was still induced by PLX4720 treatment.
Taken together, our data indicate, that Rb1 is unlikely to be responsible for the increased expression of *TGF-β2* after $BRAF^{V600E}$ kinase inhibition with PLX4720.

P.08.17

Regnase-2 reduces invasiveness of glioblastoma cells by down-regulation of metalloproteinase 2

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The Regnase/ZC3H12/MCPIP family of proteins consists of 4 members, called Regnase1-4/ZC3H12A-D/MCPIP1-4. All of them possess a highly conservative NYN/PIN and a CCCH zinc finger domains involved in the control of transcripts turnover. Regnase-2/ZC3H12B/MCPIP2 is highly expressed in the brain. During glioblastoma progression its level is reduced which correlates with poor patient prognosis.
In this study, we focused on the influence of Regnase-2 (Reg-2) on the level of metalloproteinase 2 (MMP2) in U251-MG human astrocytoma cell line. MMPs play an important role in tumor progression due to their ability to degrade the extracellular matrix. Our results indicate that Reg-2 overexpression leads to MMP2 mRNA and protein level decrease. By gelatin zymography, we also showed a decrease in the MMP2 activity in cells overexpressing Reg-2. This effect is reflected in the reduced ability of astrocytoma cells to migrate. In samples of glioblastoma from patients low level of *Reg-2* correlates with high level of *MMP2* transcripts. Our results indicate the involvement of Reg-2 in the regulation of metastatic properties of glioblastoma cells.

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P.08.18

Regnase-2 inhibits glioblastoma cell proliferation

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Regnase-2/ZC3H12B is a member of the ZC3H12 family of proteins involved in the regulation of inflammation through the degradation of proinflammatory transcripts such as IL-1β and IL-6. Endonucleolytic activity relies on the conservative NYN/PIN domain. Reg-2 is highly expressed in the brain, but its expression is reduced during glioblastoma progression and neuroinflammation. We have employed human glioblastoma cell line U87-MG to study the influence of Reg-2 on glioblastoma cell proliferation. Cell proliferation assay and cell counting experiments revealed that cells with overexpression of Reg-2 proliferate much slower than control cells. Propidium iodide staining and cytometric analysis showed that cells overexpressing Reg-2 are arrested in the G1 phase of the cell cycle. This phenomenon is NYN/PIN-dependent since much weaker effect was observed for the RNAse-inactive mutant. We demonstrated that overexpression of Reg-2 causes a decrease in the level of transcripts involved in the cell proliferation and cell cycle, namely: Cyclin E1, E2, B1, B2, A2, D1, PLK1, AURKA, and CDK1. Some of the results were also verified at the protein level. Interestingly part of the targets, were regulated in an NYN/PIN-independent manner. The results indicate that Reg-2 is a strong negative regulator of cell proliferation.

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P.08.19

Construction of a set of novel transposon vectors for efficient silencing of protein and lncRNA genes *via* CRISPR interference

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In recent years, CRISPR interference (CRISPRi) technology of gene silencing has emerged as a promising alternative to RNA interference (RNAi) surpassing the latter in terms of efficiency and accuracy. Here, we describe the construction of a set of transposon vectors suitable for constitutive or tetracycline (doxycycline)-inducible silencing of genes of interest via CRISPRi method and conferring three different antibiotic resistances, using vectors available via Addgene repository. We have analyzed the performance of the new vectors in the silencing of mouse *Adam10* and human lncRNA, *NORAD*. The empty vector variants can be used to efficiently silence any genes of interest.

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P.08.20

A novel transposon-based platform for fluorescent imaging and analysis of stress granules in eukaryotic cells

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Stress granules (SGs) are dynamic cytoplasmic complexes that contain RNA-binding proteins, transcripts, and translation initiation factors. They are an example of membrane-less organelles (MLOs), i.e. separate intracellular compartments that are not surrounded by a lipid bilayer. SGs arise in response to blocked translation initiation. SGs are thought to sequester mRNA during stress to preserve the transcriptome, allowing translation to resume once the stress conditions are over. Here, we present the development and optimization of a novel platform for the imaging and analysis of SGs in eukaryotic cells. The presented system is based on a set of newly generated and validated *Sleeping Beauty* transposon vectors allowing for constitutive, low-level expression of two well-established fluorescently labeled markers of SGs, namely G3BP1 and eIF3η. Both markers are available in fusions with three different, bright, photostable, and monomeric fluorescent proteins (green - Clover, red - mScarlet or near-infrared - miRFP670) and antibiotic resistances suitable for generation of stably transfected cell lines expressing one or two color markers simultaneously. We have analyzed and compared the performance of the generated vectors in both fixed and living cells.

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P.08.21

The use of a supramolecular carrier with a tyrosine kinase inhibitor (nilotinib) in targeted anticancer therapy of bladder cancer

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As research shows, increased activation of receptor tyrosine kinases (PDGFR, c-KIT) and the intracellular transmission pathway PI3K/AKT/mTOR may play an important role in the development of bladder cancer. For these reasons, tyrosine kinase inhibitors (e.g. nilotinib) are one of the most advanced examples of personalized medicine. However, even this therapeutic approach can prove insufficient and have adverse effects on healthy tissues. Therefore, great interest in target drug delivery is aroused by carriers with supramolecular structure, which are formed by self-association.
The aim of the study was to investigate the importance of c-KIT expression in bladder cancer, together with the in vitro evaluation of the effect of the drug alone and the drug in a complex with a carrier on bladder cancer cells.
The optimal carrier-drug molar ratio was determined, and a preliminary evaluation of complex formation was made. The experiments revealed that the investigated compounds inhibited the proliferation of bladder cancer cells. FACS analysis independently showed that tested compounds induced apoptosis.
Supramolecular systems can provide targeted delivery of therapeutic agents and may be used in the future in targeted anticancer therapy.

P.08.22

Understanding nucleolar stress management through the lens of CHIP ubiquitin ligase

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Our research explores the complex dynamics of the heat shock response, explicitly focusing on the role of the nucleolus - a unique nuclear biomolecular condensate recognized as a refuge for damaged proteins, aiding in their post-stress refolding. Central to our study is ubiquitin ligase CHIP, which moderates the cellular balance of protein folding and degradation in tandem with chaperones. We uncovered the nuanced relationship between CHIP, HSP70, and nucleophosmin (NPM1) in maintaining nucleolar proteostasis. Following heat shock, CHIP translocates to the nucleoli, remaining operative within its granular portion to fortify the NPM1 and HSP70 complex. However, excessive CHIP, much like HSP70 inhibition, impacts the availability of NPM1 and HSP70 to misfolded proteins, subsequently obstructing their elimination. Moreover, we found that CHIP depletion can reshape nucleoli morphology, obstructing post-stress ribosome formation. Our findings elucidate a new mechanism where CHIP balances nucleolar proteostasis capacity and ribosome assembly. This process, steered by CHIP regulated expression, advances our understanding of cellular stress responses.

P.08.23

P2Y2 nucleotide receptor as the regulator of glioma motility

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The nucleotide receptors, responding to extracellular nucleotides constitute an important class of cell surface receptors capable of evoking cytoplasmic signals. Extracellular nucleotides are critical but often neglected signal transmitters within the brain and the central nervous system. There are two major sources of extracellular nucleotides. The first is the activity of neurons, signaling stimulation to the astrocyte syncytium, and the second is a leak of nucleotides from the cytoplasm of injured cells. The signal coming from dying tumor cells activates microglia and initiates an in-brain form of the inflammatory response. Glioma cells are submitted to nucleotide gradients originating from active regions of the brain and later strong nucleotide signal originated by necrotic tissue resulting from the developing tumor. Gliomas are particular tumors, they are developing in the central nervous system, isolated behind the blood-brain barrier. Most tumors spread using circulation to form distant metastases, and gliomas separated from the circulation spread using cell motility and motile properties of cells are here especially important. The present study shows the effect of siRNA inhibition of the P2Y2 receptor on the motility of glial cells. We show that inhibition of the P2Y2 receptor statistically reduces the directionality and speed of C6 rat glioma cells while not influencing cell adhesion.

P.08.25

New Uracil Analog- U-359 can reverse resistance to Taxol in MCF-7 cancer cells

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Tx shows a substantial single-agent activity in metastatic breast cancer, but the main problem with its use is the development of resistance. One of the strategies used to prevent the emergence of drug resistance is combination treatment, involving at least two drugs. The aim of this study was to assess if a new uracil analog, 3-p-bromophenyl-1-ethyl-5-methylidenedihydrouracil (U-359) can prevent the development of Tx resistance in breast cancer cells. Tx co-administered with U-359 inhibited proliferation of MCF-7 cells while the level of ATPase drastically decreased, compared with effects produced by Tx alone. The apoptosis process was induced through the mitochondrial pathway. The obtained results have shown that U-359 produced a synergistic effect with Tx probably by reducing Tx resistance in MCF-7 cells. To elucidate the possible mechanism of resistance, expression of tubulin III (TUBI-II), responsible for microtubule stabilization and tau and Nlp proteins, responsible for microtubule dynamics, was assessed. The obtained results indicated that Tx+U-359 decreased the level of Nlp and TUBIII almost completely, as compared with the effect produced by Tx alone. This data suggest that analog U-359 shows significant synergistic effect in combination with Tx and can prevent the development of Tx resistance in MCF-7 cells causing alterations in microtubule dynamics and changes in microtubules' stabilization.

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