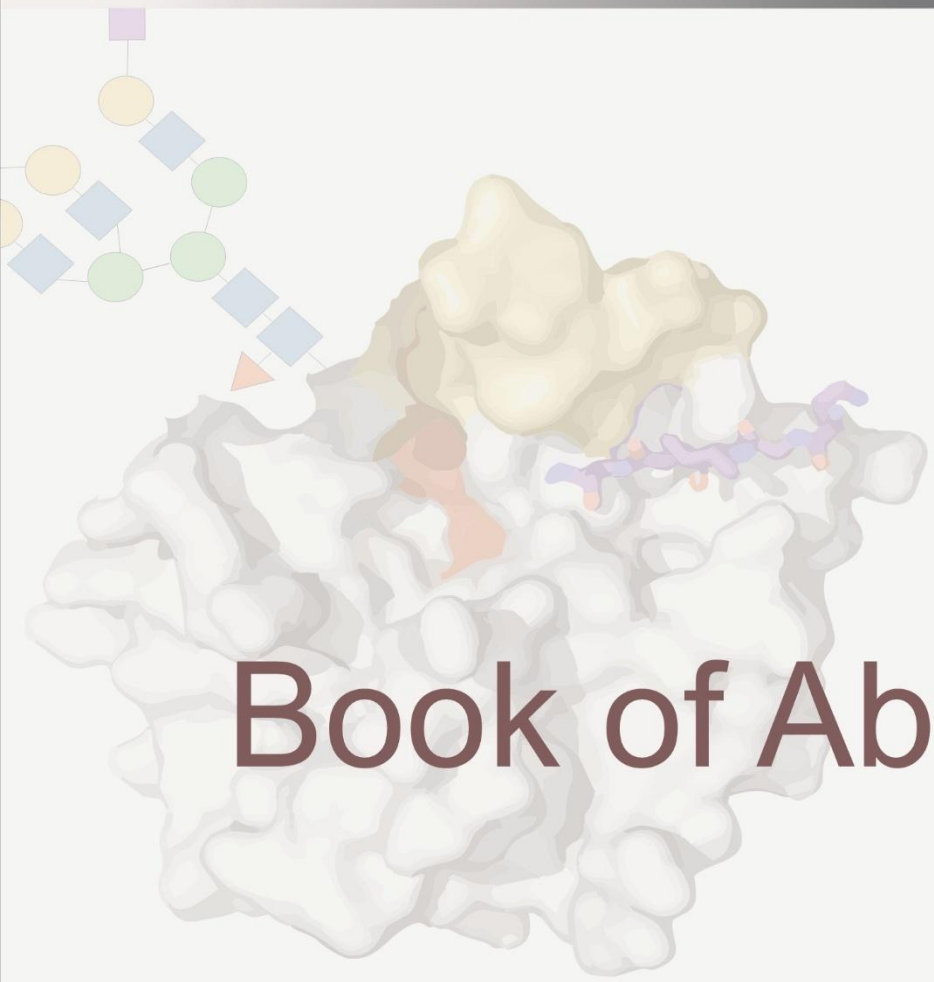
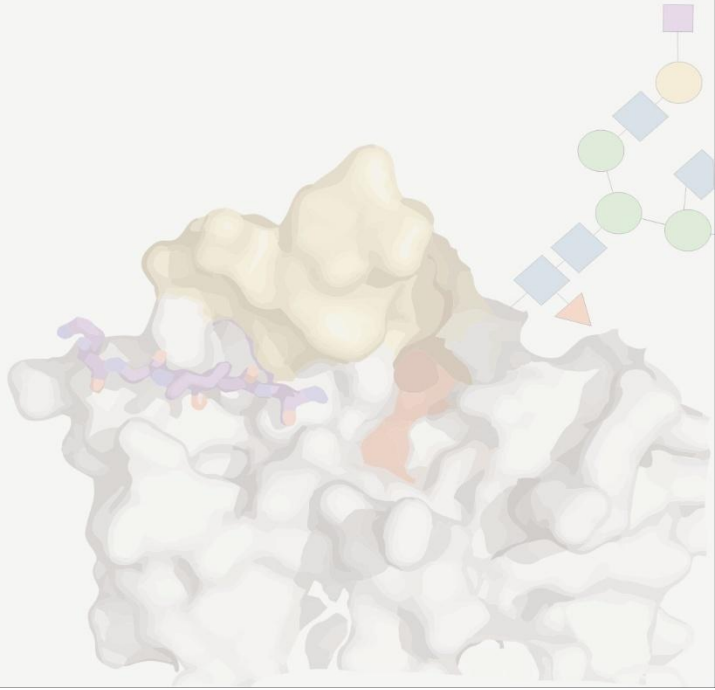
Glyco2025

5th September 2025
Kraków



Book of Abstracts



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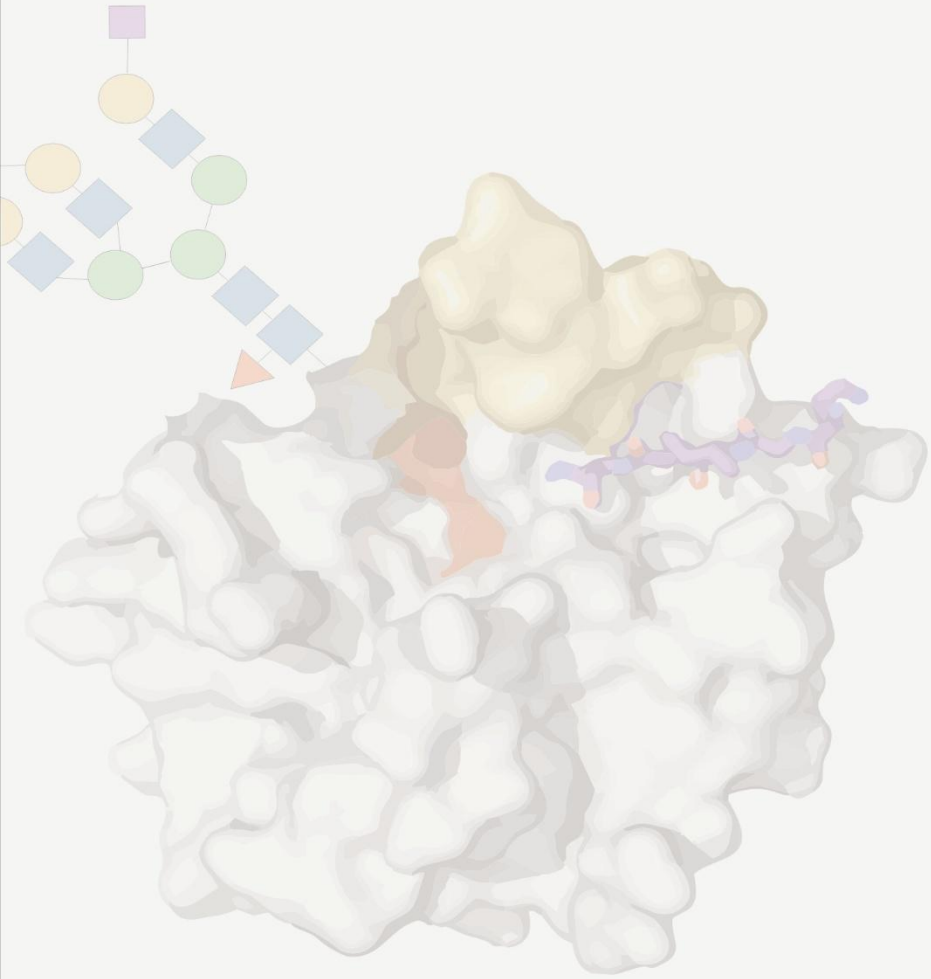
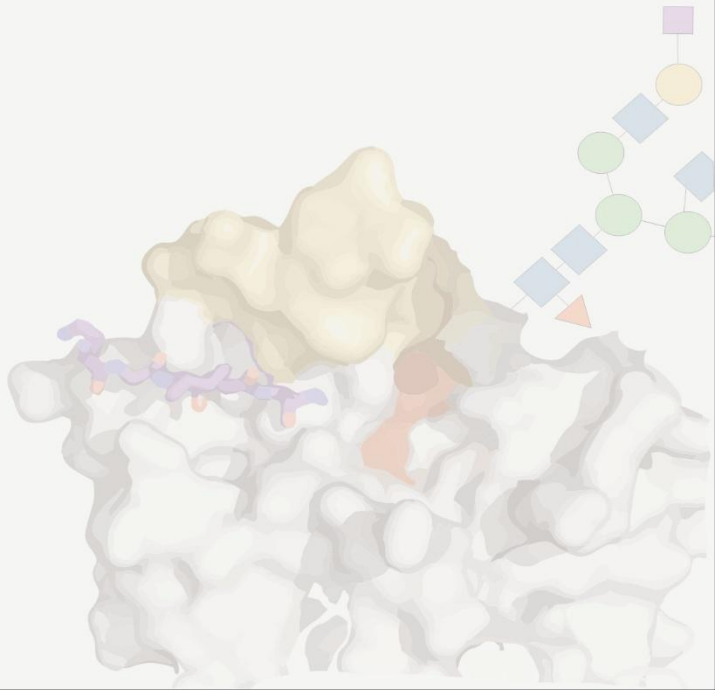


Plenary lectures



P1

Beyond Recognition: N-Glycosylation-driven strategies to enhance efficacy of CAR-T cell based immunotherapies.

Agnieszka Graczyk-Jarzynka

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Chimeric antigen receptor (CAR) T cell therapies have transformed the landscape of hematological cancer treatment, yet their efficacy in solid tumors remains modest due to the immunosuppressive tumor microenvironment (TME). A central player in this suppression is PD-L1, whose stability and receptor-binding ability critically depend on N-glycosylation. Moreover, N-glycans emerge as active regulators that can be modulated to enhance therapeutic outcomes. Pharmacological inhibitors of N-glycosylation, as well as glycan-specific antibodies, have demonstrated the potential to sensitize tumor cells to immune attack.

In this presentation, I will explore how *N*-glycosylation shapes tumor–immune interactions and how its modulation may empower CAR-T–based strategies. I will first discuss the feasibility of targeting PD-L1 glycoforms with glycan-specific antibodies and engineered conjugates. Next, I will highlight how the CAR-T secretome dynamically reshapes the tumor cell surface glycome, influencing both immunosuppression and recognition. Finally, I will present how CAR-T activation remodels the T cell N-glycome and how glycosylation modulators can be leveraged to tune CAR-T cell performance.

By integrating glycoscience with immunotherapy, this talk will outline *N*-glycosylation-driven strategies to expand the therapeutic potential of CAR-T cells in solid tumors and inspire discussion on exploiting glycobiology for next-generation cancer therapies.

P2

Insights into the evolution of the mammalian brain via comparative *N*-glycomics.

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The human brain is unique due to its remarkable cognitive abilities, yet the biological foundations of our higher-order brain functions remain largely mysterious. While various comparative “omics” studies have revealed particular human-specific features of neurobiology, the contribution of post-translational sugar modifications has not been extensively explored and, consequently, an evolutionary perspective of protein glycosylation in the mammalian brain is lacking.

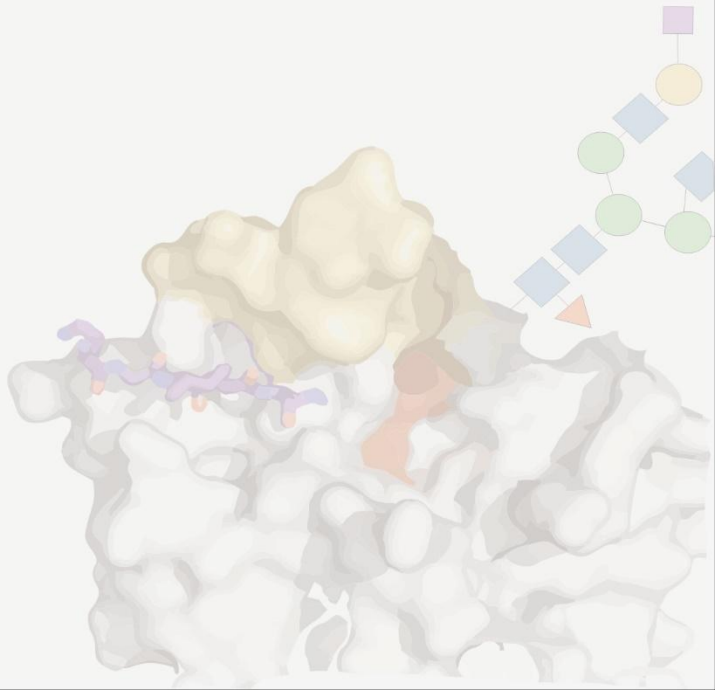
N-glycosylation is a common feature of transmembrane, secreted, and extracellular proteins that is critically involved in many aspects of neurobiology, such as synaptic transmission, membrane excitability, and neuronal plasticity. To investigate the evolutionary trajectory of *N*-glycosylation in the mammalian brain with the aim of uncovering human-specific specializations, we performed *N*-glycoprofiling across multiple brain regions from rat, macaque, chimpanzee, and human using a combination of liquid chromatography and mass spectrometry, then integrated these datasets with bulk and single-nucleus glycotranscriptomic data.

We found that, among the primate species, the brain *N*-glycomes have diverged more rapidly than the underlying transcriptomic frameworks, suggesting that N-glycosylation is a mechanism for rapidly generating interspecies diversity at the molecular level. We uncovered numerous phylogenetic trends in brain protein *N*-glycosylation as well as several human-specific adaptations. Our data suggest that brain *N*-glycome evolution in hominids has been characterized by an overall increase in complexity coupled with a shift toward increased usage of α(2-6) linked sialic acid.

Overall, our findings shed light on the evolution of the human brain N-glycome and support the notion that increased diversity and complexity of sugar modifications found on neural N-glycoproteins may have contributed to the emergence of novel cognitive functions, including those unique to the human neocortex.

Session 1

Glycobiology of cancer



O1.1

The resistance of breast cancer cells to apoptosis is increased by galactosylceramide through the EGFR signaling pathway.

Safoura Reza[1], Jarosław Suchański[1], Michał Grzybek[2], Anita Chryniewicz-Jankowska[3], **Maciej Ugorski**[1]

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Background. Our previous studies demonstrated that galactosylceramide (GalCer) acts as an anti-apoptotic molecule and increases the resistance of breast cancer (BC) cells to anticancer drugs. Consistent with these findings, we showed that GalCer downregulates the expression of the pro-apoptotic genes TNFRSF1B and TNFRSF9, while upregulating the expression of the anti-apoptotic gene BCL2 at both the mRNA and protein levels, suggesting transcriptional regulation by GalCer. One of the most likely regulatory proteins involved in this process is p53, which is known to simultaneously upregulate TNFRSF1B and TNFRSF9, and downregulate BCL2. Notably, the expression of p53 appears to be modulated by GalCer. Based on these findings, we hypothesize that GalCer may influence the apoptotic properties of BC cells by modulating specific signaling pathways.

Methodology. In the present study, we used cellular models representing both loss-of-function (MDA-MB-231 cells with knockout of the UGT8 gene and, therefore, lacking GalCer) and gain-of-function phenotypes (MCF7 cells overproducing GalCer). The identification of proteins involved in the resistance of BC cells to apoptosis was performed using Western blotting and immunofluorescence microscopy.

Results and conclusions. We have shown that the GalCer-dependent decrease in p53 expression in BC cells is most likely regulated by the MDM2 protein, which is upregulated in GalCer-positive cell lines. MDM2, in turn, is upregulated via the AKT signaling pathway, as evidenced by a marked increase in phosphorylation of AKT and PDK1 proteins in GalCer-positive cells. Furthermore, activation of the AKT signaling pathway is driven by EGFR activation in BC cells with high levels of GalCer, as indicated by increased phosphorylation of EGFR. Since GalCer is localized in lipid rafts, we propose that raft-associated GalCer activates EGFR in a ligand-independent manner. However, the molecular mechanism of EGFR activation by GalCer, whether via direct glycosphingolipid-protein interaction or via indirect glycosphingolipid-mediated reorganization of lipid raft structure, remains to be elucidated.

O1.2

Rational control of proteoglycans endocytosis– towards upgraded precision and efficiency of protein-drug conjugates delivery into cancer cells.

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Background. Protein drug conjugates (PDCs) are targeted anticancer therapeutics, composed of tumor marker recognition particle covalently coupled to a cytotoxic drug. Precise identification of the tumor marker by PDC and rapid efficient endocytosis of the receptor/PDC and its lysosomal delivery constitute critical steps for PDC activity. Novel approaches that improve PDC targeting are highly needed to overcome limitations of current PDCs and enable targeting of novel oncoproteins. Heparan sulfate proteoglycans (HSPGs) are extracellular matrix (ECM) components contributing to cancer development, especially pancreatic cancer, yet selective therapies targeting HSPGs are lacking.

Methodology. We used protein engineering, biophysical, biochemical and cell biology approaches, high content screening (HCS) microscopy with extensive compound library.

Results and conclusions. We developed the first PDC selective for heparan sulfate (HS) part of HSPGs. We demonstrate that due to multivalency our PDC triggers ultrafast and highly efficient aggregation-dependent endocytosis (ADE) of HSPG/PDC and their rapid lysosomal delivery, where cytotoxic drug is released. Endocytosis of cell surface receptors is under the tight control of intracellular signaling pathways, yet signaling cascades that control ADE were largely unknown. We employed high content screening (HCS) with automated microscopy and near kinome-wide library of kinase inhibitors to identify ADE regulators. We identified pathways that modulate cellular uptake of multivalent PDC. Furthermore, we performed subsequent HCS selections that allowed us to determine inhibitors, which blocked endocytosis of multivalent PDCs into heathy cells and simultaneously upregulated their internalization into corresponding cancer cells. Finally, priming cells with endocytic modulators allowed for improvement of the selectivity of treatment with PDC. Our approach enables targeting of novel cancer receptors, so far excluded from targeted therapies due to low endocytosis or minor difference in expression level between normal and cancer cells.

This work was supported by OPUS grant (2021/43/B/NZ1/00245) from the National Science Centre.

O1.3

N-glycosylation as a modulator of functionality of extracellular vesicles and its diagnostic potential in bladder cancer.

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Background. Transport of bioactive molecules through extracellular vesicles (EVs) is one of the mechanisms of intercellular communication. EVs regulate a number of physiological processes in recipient cells, and also contribute to development and progression of many diseases, including cancer. Many glycoproteins are incorporated into EVs, but little is known about their biological significance. Glycosylation can affect protein sorting into EVs and the interaction of EV with the target cell. Therefore, glycan contents of EVs are being studied for the if possible diagnostic and therapeutic use.

Methodology. Structural analysis and identification of N-glycans differentiating EVs from normal (HCV-29) and cancerous (T-24) bladder cells was performed using MALDI-TOF. Lectin-based flow cytometry staining was carried out to characterize surface glycosylation of EVs. Finally, it was investigated whether the glycosylation status is responsible for the effect exerted by EVs on the proliferation (AlamarBlue assay) and migration (Wound Healing assay) of various types of recipient cells.

Results and conclusions. MALDI-TOF identified a total of 38 N-glycan structures in EVs from both cell lines, and the most abundant structures included paucimannose and triantennary sialilated and fucosylated N-glycans. Respectively, 20 and 8 structures were unique for HCV-29 and T-24 EVs, whereas 21 structures displayed different abundance between HCV-29 and T-24 EVs. EVs also displayed distinct surface glycosylation profiles with abundance of β-1,6-branched glycans and sialilated structures. Finally, stronger proproliferative and promigratory activity of T-24-derived EVs was observed in comparison to EVs from HCV-29 cells. When EVs were isolated from cells treated with glycosylation inhibitors, the aforementioned effects were diminished, suggesting that glycans carried by EVs were involved in modulation of recipient cell function. Obtained results provide preliminary insight into the structural diversity and biological significance of N-glycans present in EVs derived from bladder cancer cells. Its promising findings justify further, more in-depth research on EV N-glycosylation.

O1.4

Overexpression of the B3GALT1 gene and its impact on protein expression and cell behaviour.

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Background. A unique set of sugar antigens presented on the surface of a cancer cell affects its survival, migration, invasiveness, and metastatic potential. The expression of the oncofetal Lewis C antigen, which is synthesized by β1,3 GalT1 and encoded by the B3GALT1 gene, increases during the neoplastic transformation of melanocytes. Unlike melanoma cells from the vertical growth phase (WM793), metastatic melanoma cells (WM266-4) exhibit a non-synonymous point mutation (c.C164G) in the B3GALT1 gene. The goals of the research were to: 1) investigate the impact of the Lewis C antigen on the proliferation, migration, and invasion of transfected normal epithelial cells, and 2) identify proteins deregulated by B3GALT1 gene overexpression using quantitative analysis.

Methodology. The research model used CHO-Lec2 cells that had been transfected with an empty vector or a vector containing either a non-mutated or mutated human B3GALT1 gene. We evaluated the functional effects of B3GALT1 overexpression in terms of cell proliferation (AlamarBlue assay), migratory properties (wound healing assay), and invasive properties (3D invasion assay). Deregulated proteins in CHO-Lec2 cells overexpressing B3GALT1 were identified by mass spectrometry and analyzed using Gene Ontology.

Results and conclusions. A decline in the proliferation and migration rates of CHO-Lec2 cells overexpressing the B3GALT1 gene was observed. Overexpression of the B3GALT1 gene triggers the invasive potential of individual CHO-Lec2 cells. These cells adopt a mesenchymal shape and can infiltrate the extracellular matrix. CHO-Lec2 cells with a non-mutated B3GALT1 gene had 222 deregulated proteins, primarily involved in mRNA translation. In contrast, CHO-Lec2 cells with the mutated gene had 54 deregulated proteins, predominantly engaged in protein glycosylation. The B3GALT1 gene mutation deregulates only six genes involved in DNA repair and the response to stress.

The research has been supported by grants from the Faculty of Biology under the Strategic Programme Excellence Initiative at Jagiellonian University and National Science Centre, Poland (2016/21/B/NZ3/00348).

O1.5

UGT8/GalCer-Dependent Resistance of Breast Cancer Cells to Drug-Induced Apoptosis is Regulated by the LIM/Homeobox Protein LHX6.

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Background. We previously demonstrated that overexpression of ceramide galactosyltransferase (UGT8) in breast cancer (BC) is associated with an increased risk of lung metastasis. UGT8 catalyzes the synthesis of galactosylceramide (GalCer), which promotes BC cell survival in the tumor microenvironment and enhances resistance to chemotherapeutic agents via anti-apoptotic mechanisms. This study aimed to identify the molecular mechanisms responsible for UGT8 overexpression in aggressive BC cells.

Methodology. The UGT8 gene promoter region and a series of deletion constructs were generated by PCR and analyzed using the Dual-Luciferase Reporter Assay System (Promega). Functional regulatory elements were identified via electrophoretic mobility shift assays (EMSA) using biotin-labeled oligonucleotides and nuclear extracts. Candidate transcription factors were predicted using the JASPAR database. Expression of UGT8 and transcription factor was assessed by qPCR, Western blotting, and immunohistochemistry. GalCer levels were determined by thin-layer chromatography binding assays. LHX6-DNA interactions were evaluated by surface plasmon resonance (SPR). Apoptosis was measured using the Thermo Dead Cell Apoptosis Kit.

Results and conclusions. UGT8 gene promoter activity was significantly higher in MDA-MB-231 cells than in UGT8-negative T47D and MCF-7 cells, suggesting the presence of enhancer elements. A key regulatory region (-1132 to -1618 bp), designated as the UGT8 response element (UGT8RE), was identified. In silico prediction and expression profiling identified LHX6 as a potential regulator. Site-directed mutagenesis confirmed the importance of the LHX6BS2 site for promoter activity. RNAi-mediated silencing of LHX6 led to downregulation of UGT8 and GalCer, sensitizing cells to doxorubicin-induced apoptosis. These findings establish LHX6 as a crucial regulator of UGT8 gene expression and a promising therapeutic target in drug-resistant BC with elevated UGT8 expression.

Grant support for this work was provided by the National Science Center, Poland (Grant no. 2019/35/B/NZ5/01392).

O1.6

Tunicamycin-related deregulation of uveal melanoma cells – a proteomic point of view.

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Background. Tunicamycin (TN) is known as an N-glycosylation inhibitor. It induces the accumulation of misfolded proteins in the endoplasmic reticulum (ER) and the unfolded protein response (UPR). Our aim was to demonstrate to what extent TN affects protein expression in uveal melanoma cell lines: 92.1 and Mel202.

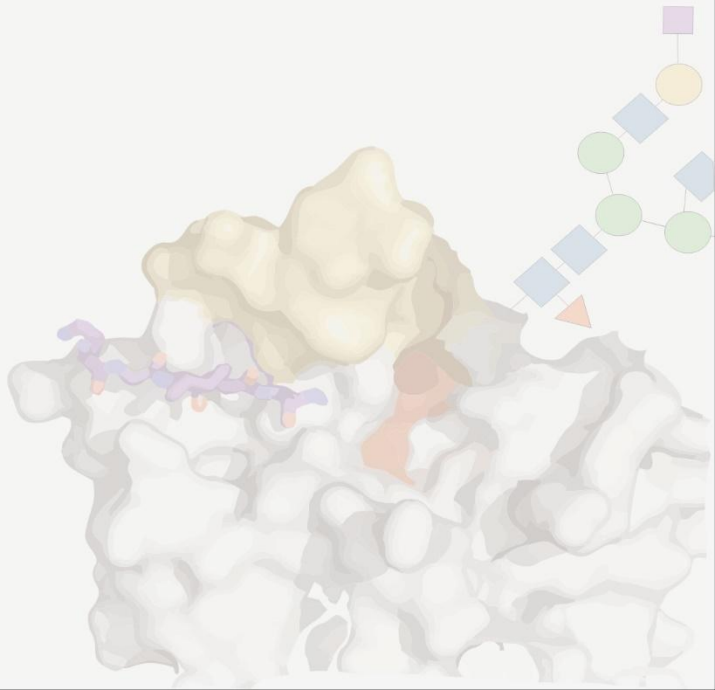
Methodology. We identified proteins that were uniquely expressed or deregulated under TN treatment using mass spectrometry and analysed them according to the Gene Ontology database.

Results and conclusions. Mel202 cells were shown to exhibit a more pronounced deregulation of their protein expression profile in response to TN treatment compared to 92.1 cells. Analysis of the functional protein association network of 92.1 cells showed that the up-regulated proteins were more networked than the down-regulated. In 92.1 cells, the down-regulated proteins were mainly associated with mesenchymal migration, while the unique and up-regulated proteins were involved in UPR, ER stress and integrin signalling. In Mel202 cells, down-regulated proteins were generally attributed to the citric acid cycle and respiratory electron transport, but small clusters related to the glycosylation process and integrin activity were also identified. In this cell line, the unique and up-regulated proteins were associated with translation regulation, with a small cluster of proteins involved in detoxification. The proteins most up-regulated in 92.1 cells were laminin subunits, annexins and HSPA5, and in Mel202 cells it was the translation regulator LARP1. TYRP1 was significantly down-regulated in both cell lines, whereas TYR, also associated with melanin synthesis, was down-regulated only in Mel202 cells. TN action pushes UM cells to restore homeostasis, but through different mechanisms. In addition, both cell lines were shown to express chemoresistance-related proteins.

Work supported by a grant from the National Science Centre, Poland (2021/05/X/NZ3/00696).

Session 2

Glycoproteins and glycosylation pathways



O2.1

SLC35A2 deficiency disturbs Golgi homeostasis in a galactosylation-independent fashion.

Dorota Maszczak-Seneczko, Natalia Molsa, Oleksandr Zalutskyi, Wojciech Wiertelak, Mariusz Olczak

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Background. SLC35A2 is the only known UDP-galactose transporter found in the endoplasmic reticulum (ER) and Golgi membranes of mammalian cells. Over a decade ago, we demonstrated that this multitransmembrane protein interacts with several glycosylation enzymes. Since then, the range of SLC35A2 interaction partners has significantly expanded, now including glycosyltransferases involved in the biosynthesis of N-glycans (Mgats 1-5), O-glycans (C1GalT1), glycosaminoglycans (B4GalT4), and proteins that help maintain Golgi homeostasis, such as the Golgi pH regulator B and the Golgi calcium/manganese-proton antiporter TMEM165. Recently, we demonstrated that SLC35A2 deficiency leads to decreased protein levels and impaired Golgi localization of C1GalT1 and its chaperone, Cosmc. As a continuation of this study, we analyzed the protein levels of selected glycosylation enzymes and some other proteins relevant to Golgi function in SLC35A2-deficient cells in comparison with cells with disrupted UDP-galactose synthesis due to a deficiency in UDP-galactose 4-epimerase (GALE). This approach allowed us to rule out secondary effects associated with impaired galactosylation, as neither SLC35A2- nor GALE-deficient cells incorporate galactose into glycoconjugates.

Methodology. We performed Western blot analyses on whole cell lysates using specific antibodies and conducted densitometric analysis with normalization to GAPDH or Ponceau S.

Results and conclusions. We observed alterations in the levels of several glycosylation enzymes, including B3GalNT1, C1GalT1, MAN1A1, and UGGT1 in SLC35A2-deficient cells compared to GALE-deficient cells. Furthermore, the levels of GM130 (a cis-Golgi matrix protein) and TFE3 (a transcription factor mediating one of the Golgi stress response pathways) were significantly reduced in the SLC35A2 knockout compared to the GALE knockout. Additionally, TMEM165 derived from SLC35A2-deficient cells migrated more slowly in SDS-PAGE compared to that from GALE-deficient cells. Therefore, it appears that SLC35A2 depletion has a broad and profound impact on the glycosylation machinery and Golgi homeostasis, which occurs independently of the compromised galactosylation.

O2.2

The expression of SLC35C1 fucose transporter in peripheral blood mononuclear cells (PBMCs) differs between patients with Hashimoto's thyroiditis and healthy donors.

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Background. Fucose (Fuc) is a monosaccharide whose attachment to glycans is catalyzed by fucosyltransferases (Futs). All Fut enzymes use a nucleotide-activated form of Fuc (GDP-Fuc) as a substrate for the formation of fucosylated oligosaccharides. GDP-Fuc is transported to the Golgi apparatus (GA) by a nucleotide sugar transporter belonging to the soluble carrier family 35C1 (SLC35C1). Fucosylated glycans are involved in physiological and pathological processes, including autoimmunity. Peripheral blood mononuclear cells (PBMCs) play a key role in Hashimoto's thyroiditis (HT). We have demonstrated an increased fucosylation of T helper (Th) cells in HT compared to healthy donors. Oligosaccharide structure is strictly dependent on the expression of the nucleotide sugar transporter. Therefore, this study aimed to analyze the gene expression and protein content of SLC35C1 and its localization in the Golgi apparatus of PBMC cells from patients with HT and the control group.

Methodology. PBMCs from HT patients and healthy individuals (CTR) were isolated from blood using a density gradient on Histopaque-1077. The isolated cells were then cultured in vitro for 24 hours with selected cytokines. Western blot and RT-qPCR analyses were performed to determine whether the content of the SLC35C1 transporter changes at the protein and gene levels. In addition, the localization of SLC35C1 in the cis and trans GA cisternae was examined using a confocal microscope.

Results and conclusions. Analysis of the data obtained for Western Blot and RT-qPCR showed a significantly higher content of SLC35C1 protein and its mRNA transcript in PBMCs from HT patients compared to CTR. Colocalization analysis confirmed the higher SLC35C1 content in HT PBMCs compared to healthy individuals in both GA cisternae. Interestingly, all tested cytokines reduced the mRNA level for the fucose transporter in the HT group compared to unstimulated cells. The results provide further data important to understand the changes of Th cell fucosylation in HT.

This study was supported by the BioS Priority Research Area under the "Initiative of Excellence Research University" program at Jagiellonian University in Krakow (No. U1U/W18/NO/28.56).

O2.3

Insights into human alpha1,4-galactosyltransferase dimerization: the role in enzyme acceptor specificity and interactions with Shiga toxins.

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Background. Human alpha1,4-galactosyltransferase (Gb3/CD77 synthase, A4galt), encoded by the A4GALT gene, is a Golgi apparatus-resident glycosyltransferase (GT) that catalyzes the attachment of Gal residues to glycosphingolipid (GSL) and glycoprotein (GP) acceptors, forming Galα1→4Galβ1→4Glc-Cer (Gb3) and Galα1→4Galβ1→4GlcNAcβ1→R (P1 glycotope), respectively. These structures serve as receptors for Shiga toxin type 1 (Stx1) and type 2 (Stx2), produced by Stx-producing Escherichia coli (STEC). The dual acceptor specificity of human A4galt may result from interactions with other GTs involved in proximity glycosylation pathways. Our efforts aimed to elucidate the ability of human A4galt to form homo- and heterodimers with other GTs and to determine the role of heterodimerization in the distribution of Stx-related receptors on the surface of Chinese hamster ovary (CHO-Lec2) cells.

Methodology. A NanoBiT-based assay was used to investigate the ability of human A4galt to homo- and heterodimerize with GSL-specific β1,4-galactosyltransferase 5 (B4galt5) and GP-specific β1,4-galactosyltransferase 1 (B4galt1). CHO-Lec2 cells expressing human A4galt were genetically modified using CRISPR/Cas9 technology to knock out endogenous hamster B4GALT5, encoding GSL-specific hamster B4galt5.

Results and conclusions. Human A4galt forms both homodimers and heterodimers with human B4galt1 and B4galt5. Structural modeling using AlphaFold predicted the highest confidence for A4galt-B4galt5 heterodimers, confirming the preference of A4galt for GSL-based acceptors. Although B4GALT5 KO CHO-Lec2 cells lacked the Gb3 GSL, these cells retained the ability to synthesize GP-based P1 glycotopes and exhibited resistance to Stx-induced cytotoxicity. These findings indicate that GP-based receptors may act as decoys for Stx. Moreover, this study offers novel insight into the dual substrate specificity of A4galt and highlights the potential of P1 glycotopes as therapeutic candidates for the mitigation of Stx-mediated diseases.

This research was funded by the National Science Centre of Poland, PRELUDIUM 20 Project 2021/41/N/NZ6/00949.

O2.4

Modelling Glycosylated Neurotransmitter Receptors reveals Glycan-mediated Constraints on Ion Conduction.

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Background. Neurotransmitter receptors are fundamental to synaptic communication, yet their function is often computationally studied in simplified models that neglect post-translational modifications like glycosylation. The functional role of native glycans is often ignored in pharmacological research as a result, leading to a potentially flawed understanding of signalling mechanisms and inaccurate conclusions about receptor pharmacology. Here, we provide a comprehensive pipeline for glycan-aware computational modelling of neurotransmitter receptors, with an initial focus on the clinically significant GABAA receptor.

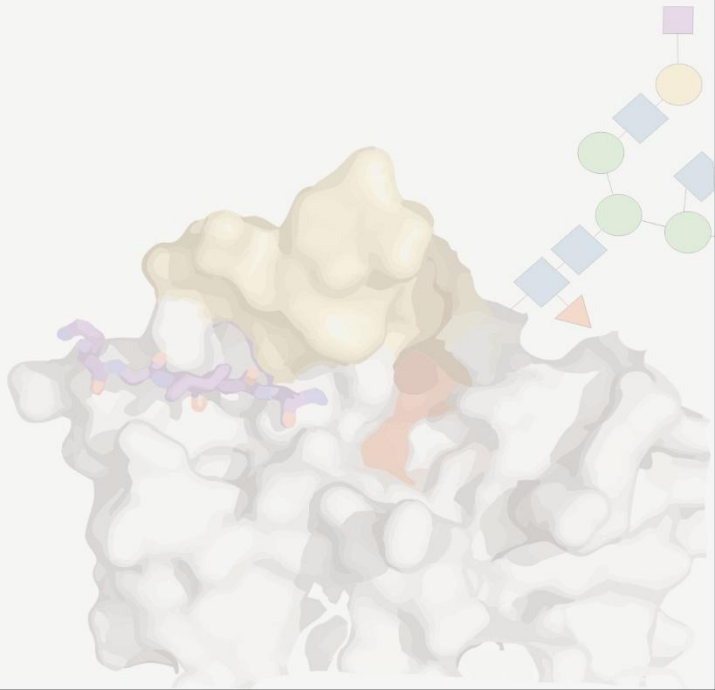
Methodology. A computational pipeline integrating homology modelling, our in-house tool for glycan shielding prediction: GlycoSHIELD, and molecular dynamics simulations was developed to construct a glycosylated model of the α1β3γ2 subtype GABAA receptor. Using microsecond-scale molecular dynamics simulations, we examined how glycans affect ion entry in the receptor vestibule, with particular focus on glycoforms linked to neurological diseases.

Results and conclusions. In our simulations, we observe N-glycans extending into the vestibule (inner glycans) constrict the ion conduction pathway, affecting the mean first passage time for the chloride ion to enter, and the ability of glycans to block ionic current seems to depend on their conformation, with native glycan position seen in cryoEM being most permissive. We also observed changes in protein surface shielding by glycans near ligand and drug binding sites, which may directly affect drug affinity, aligning with research on modified glycans and neurological disorders.

Our findings emphasise that overlooking glycosylation leads to an incomplete and potentially incorrect view of neurotransmitter receptor pharmacology. A systematic examination of additional clinical targets, including glycine, AMPA, and NMDA receptors, is currently in progress to build an accurate understanding of synaptic signalling.

Session 3

Infection, microorganisms, evolution



O3.1

Sialic acid specificity of *Laverania* Erythrocyte Binding Antigen 140 (EBA-140).

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Background. Plasmodium parasites within the Laverania subgenus infect both humans and African great apes, and are largely host-specific. The factors that control host-specificity are likely to be multi-factorial, but the ability of merozoites to distinguish between erythrocytes through the specificity of invasion ligands is thought to be one possible mechanism. The Erythrocyte Binding Antigen-140 (EBA140) ligand belongs to the erythrocyte binding-like (EBL) protein super family and the genomes of all Laverania species contain an EBA140 orthologue. The P. falciparum EBA140 ligand has been shown to bind to the erythrocyte sialoglycoprotein Glycophorin C, while P. reichenowi ligand has been shown to recognize its truncated form Glycophorin D, both in a sialic acid dependent manner.

Methodology. Using the CRISPR-Cas9 method, we created transgenic P. falciparum lines in which PfEBA140 was exchanged with EBA140 from another Laverania parasite, P. praefalciparum, which is considered the closest ancestor of P. falciparum but infects gorillas rather than humans. The modified protein was colocalized using IFA and the lines were subsequently used in invasion and erythrocyte binding assays.

Results and conclusions. We observed significant differences in growth between transgenic and wild-type lines, but the P. praefalciparum EBA-140 line exhibited similar behavior to that of the EBA-140 knock-out line. There were no differences in invasion among the compared lines. We showed that EBA-140 is not a factor determining host specificity and its role in Plasmodium invasion can be replaced by other ligands.

O3.2

Reconstructing the glycobiology of first animals and multicellularity using comparative glycomics.

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Background. Self vs. non-self cell recognition and division of labor between groups of cells are key requirements of animal multicellularity. In mammals, N-glycosylation is tissue-specific and cancer cells are known to have altered glycosylation patterns. However, there is little N-glycomics data available from early branching animals and unicellular protists closely related to animals, leaving the evolutionary patterns in the dark.

Methodology. We conducted a systematic, comparative N-glycomics study that includes representatives of early branching animal groups (ctenophores, sponges, placozoans and cnidarians), as well as their closest protist relatives from the group Holozoa (filastereans, choanoflagellates and ichthyosporeans) and of additional eukaryotic outgroups. We also created a proteomic database to make Holozoan unicellular-to-multicellular transition studies more accessible and used it to conduct phylogenomics targeted towards finding the origins of glycosyltransferases and related genes.

Results and conclusions. We report a huge variety of N-glycan structures, including novel compositions. The data suggest that N-glycan complexity is positively correlated with organismal complexity and linked to lifestyle. Ichthyosporeans, which have a complex life cycle and often are animal parasites, synthesize a wide variety of N-glycan structures, similar to animals. In contrast, facultatively multicellular protists (C. owczarzaki, S. rosetta) synthesize simpler oligomannose N-glycans, despite possessing the genes encoding for glycan branching. Our results indicate that the N-glycan biosynthetic pathway became more important for obligate multicellularity, both as a mechanism of protein quality control and a way to synthesize recognition tags. This study provides a foundation for future work on non-canonical species by establishing several reference stage-specific glycomes. Going forward, single species-focused studies are needed to unravel the significance of the observed structures for each organism.

O3.3

Glycosylation variability of serum alpha-1-acid glycoprotein in the context of developing inflammation in patients with severe COVID-19 and convalescents after SARS-CoV-2 infection.

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Background. COVID-19, caused by coronavirus SARS-CoV-2, in 20% of patients develops symptoms of lower respiratory tract involvement and multi-organ complications. The mechanisms of the immune response are the main factor disrupting the proper functioning of the patient's body. Alpha-1-acid glycoprotein (AGP), a protein with multidirectional immunomodulatory and protective effects, contains 45% N-glycans in its molecule, and the qualitative and quantitative changes in their composition depend on the pathophysiological state of the body. The study aimed to determine serum AGP concentrations in 87 patients with severe COVID-19, 48 convalescents, and 65 healthy individuals, and to analyse the degree of sialylation and fucosylation of AGP glycans. Moreover, we planned to check whether there are significant differences between the examined groups in the values of tested parameters, analyze the correlations between their values, and determine their clinical usefulness.

Methodology. AGP concentrations were measured using the Konelab20i® biochemical analyzer. The profile and degree of AGP N-glycosylation were analysed using a lectin-ELISA method with sialo-specific lectins from Sambucus nigra (SNA) and Maackia amurensis (MAA) detecting terminal sialic acid (SA) α2,6- and α2,3-linked, respectively; fucose-specific LTA (Lotus tetragonolobus agglutinin) and AAL (Aleuria aurantia lectin) detecting antennary fucose α1,3-linked.

Results and conclusions. AGP concentrations were significantly higher in COVID-19 patients than in convalescents and healthy individuals. The degree of AGP α2,6-sialylation was significantly reduced in COVID-19 patients compared to the other study groups. The relative reactivity of AGP with LTA detecting fucose of LewisX structures was significantly reduced in the COVID-19 patients compared to the control group and convalescents. In summary, among the analyzed parameters, the highest clinical/diagnostic value has AGP concentration as well as expression on AGP glycans of SNA-reactive SA and LTA-reactive fucose, which differentiates COVID-19 patients from other groups. The expression of SNA-reactive SA is also a marker that significantly differentiates convalescents from healthy individuals. The observed very high/high negative correlations between AGP reactivities with SNA and LTA additionally confirm that with the increase in serum AGP concentration in the course of severe COVID-19, the expression of α2,6-linked terminal SA and fucose of LewisX structures on this glycoprotein decreases. The above parameters may therefore constitute promising additional serum markers of severe COVID-19.

O3.4

Role of LPS structure in the biological activity of OMVs from *Yersinia enterocolitica*.

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Background. OMVs are vesicles secreted by Gram- bacteria and are involved in the horizontal gene transfer, biofilm formation, killing of competing microbial cells, resistance to antibiotics, adherence to host cells and immunomodulation. The major component of the OMV's surface is the lipopolysaccharide (LPS). Yersinia enterocolitica O:3 (Ye O:3) is able to grow in a wide range of temperature (4-45°C). It synthesizes LPS with an unique arrangement, where the inner core (IC) is substituted with an O-specific polysaccharide (OPS) or an outer core oligosaccharide (OC). Like other Ye virulence factors, LPS expression is controlled by temperature with diminished expression of OPS at higher one. We investigated the LPS structure-dependent biological activity of OMVs.

Methodology. We investigated OMVs secreted by serum-sensitive (virulence plasmid-cured) YeO3 wild type (YeS) bacteria grown at 4°C, 22°C and 37°C. Furthermore, OMVs from three variants expressing LPS of Ra (YeRa), Rd1 (YeRd1) and Re (YeRe) chemotypes, cultivated at 37°C, were tested. OMVs were isolated from sterile culture supernatants and their concentrations and size were analysed by NTA. The capacity of OMVs to activate the complement system was tested by ELISA. OMVs labelled with DiO (fluorescent dye) were used to observe their uptake by epithelial cells using confocal and fluorescent microscopy. Different inhibitors of endocytosis were tested to identify which way(s) OMVs-DiO use to be internalized and if the way chosen depends on the LPS structure and/or size. The level of uptake was determined by flow cytometry. Biodistribution was analyzed with OMVs labelled with DiD (fluorescent dye) injected (ip.) in mice BALB/c.

Results and conclusions. Our result suggests that the LPS chemotype can affect the vesiculation of OMVs (concentration and size), their capacity to activate the complement system, the endocytosis way used in host's cells and their biodistribution over time.

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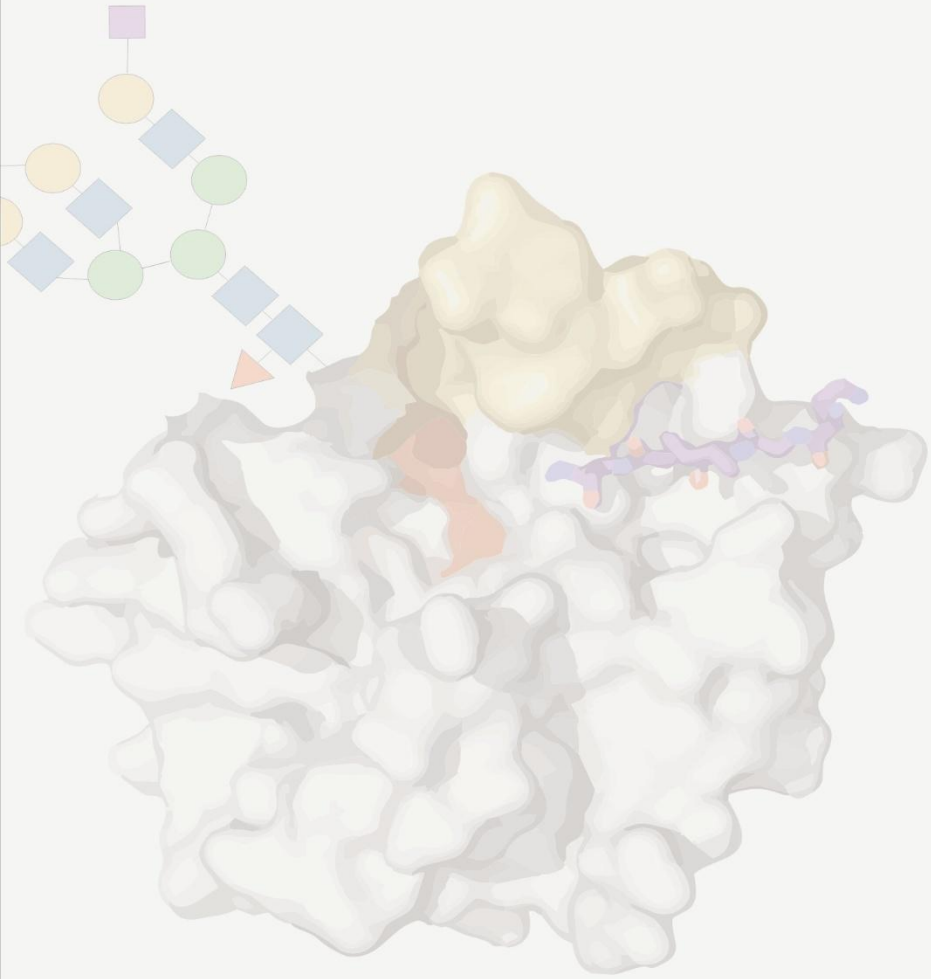
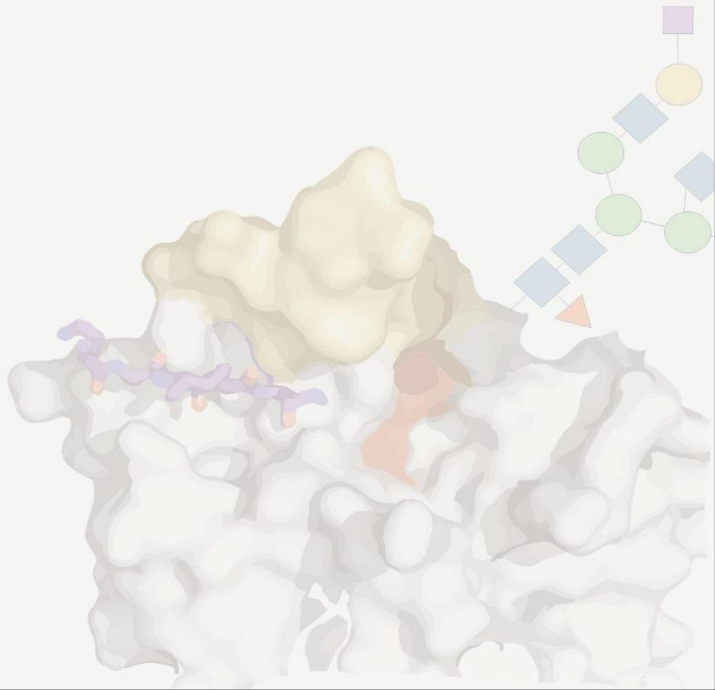


Poster Session



PS1

Remodeling of Glycan Profiles in Metformin-Treated Endothelial Cells Under Flow Conditions: A Pilot Study.

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Background. Metformin belongs to hypoglycemic drugs used in the treatment of type 2 diabetes. The mechanism of metformin's effect on different types of tissue and cells is not fully understood. Shear stress is a mechanical force that significantly influences cellular physiology, structural properties, and function. Both analyzed factors can influence the endothelial glycocalyx. Therefore, the aim of the study was to evaluate the remodeling of the glycan profile of metformin treated endothelial cells under the flow treatment.

Methodology. Shear experiments on EA.hy926 cells treated with 1 mM/ml metformin were conducted using a system consisting of a peristaltic pump and µ-Slide I0.6 Luer plates. A shear stress of 10 dyn/cm^2 was applied for 1 hour and 24 hours, and results were compared to static controls. Glycan profiles of the treated cells under flow conditions were analyzed in comparison to controls using MALDI-MS, immunofluorescence imaging, and RT-qPCR to assess the expression of enzymes involved in glycosylation pathways. Additionally, Hematoxylin and Eosin (H&E) staining, as well as Periodic Acid-Schiff (PAS) staining combined with Alcian Blue, were performed on both metformin-treated and untreated EA.hy926 cells.

Results and conclusions. Metformin activity at different doses induced morphological changes in the cells. Ultimately, a concentration of 1 mM/ml was selected for treatment. MALDI-MS analysis revealed remodeling of the N-glycan profile in metformin-treated cells following exposure to flow conditions. Flow conditions further contributed to cellular glycan remodeling and altered the expression of enzymes involved in glycosylation in metformin-treated and flow-exposed cells. Histological staining indicated changes in polysaccharide content in drug-treated cells.

The observed alterations in glycan profiles in EA.hy926 cells treated with metformin and subjected to flow may impact endothelial cell function and integrity, as well as the pharmacokinetics of metformin. These preliminary findings warrant further investigation and validation.

The project was supported by Excellence Initiative-Jagiellonian University (no. U1C/P04/NO/01.03 to MBL).

PS2

Inhibition of galactosylceramide synthesis abolishes the resistance of mammary carcinoma tumors to doxorubicin therapy.

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Background. Previous studies have demonstrated that the accumulation of galactosylceramide (GalCer) in breast cancer (BC) cells, resulting from the overexpression of galactosylceramide synthase (UGT8), confers increased resistance to the cytotoxic effects of anticancer drugs compared to their GalCer-negative counterparts (Suchanski et al., 2024). Drug resistance remains a significant obstacle in cancer therapy, particularly in patients with advanced-stage disease. Accordingly, in addition to efforts aimed at developing more effective chemotherapeutic agents, considerable research has been devoted to the development of compounds capable of reversing drug resistance. These agents, commonly referred to as drug resistance modulators, are not chemotherapeutic agents themselves but can restore drug sensitivity in otherwise resistant cancer cells when administered in combination with chemotherapy. Given that drug resistance continues to present a major obstacle in breast cancer therapy, we propose the use of small-molecule inhibitors targeting UGT8 to suppress GalCer biosynthesis, thereby enhancing the sensitivity of BC cells to conventional chemotherapeutic agents.

Methodology. We previously demonstrated that murine 4T1 mammary carcinoma cells overexpressing murine UGT8 (UGT8a) exhibit increased resistance to doxorubicin (DOX), similar to human BC cells, in contrast to control 4T1 cells lacking UGT8 expression (Suchański et al., 2024). As proof of concept for the hypothesis that UGT8 inhibition can sensitize BC cells to chemotherapeutic agents, we treated 4T1.UGT8a cells with DOX both in vitro and in vivo, either alone or in combination with a free or liposomal formulation of the UGT8 inhibitor 3-(methylcarbamoyl)-7-(trifluoromethyl)thieno[3,2-b]pyridin-5-yl)-piperidin-4-yl-(S)-1-(trifluoromethoxy)propan-2-ylcarbamate (Inhibitor 19; Thurairatnam et al., 2020).

Results and conclusions. Our results demonstrated that the liposomal formulation of Inhibitor 19 significantly enhanced the antitumor efficacy of DOX in DOX-resistant, GalCer-producing mammary carcinoma cells overexpressing UGT8. This effect is attributed to inhibition of GalCer biosynthesis, as GalCer functions as an anti-apoptotic lipid mediator (Suchański et al., 2024).

PS3

Analysis of semen plasma reactivity in men with idiopathic infertility with galectin-3.

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Background. Male infertility is mostly associated with abnormal semen characteristics. However, there is a large group of infertile men whose semen meets the WHO criteria (normozoospermic). In these cases, referred to as idiopathic infertility, the cause of reproductive failure is difficult to explain. It is assumed that some of these cases may be due to abnormal protein glycosylation, which disrupts the immunomodulatory effects of endogenous lectins, including galectin-3. The aim of this project was to analyze the Gal-3 interactome in the seminal plasma of men with normozoospermic idiopathic infertility.

Methodology. Reactivity of SP glycoproteins from four groups of infertile patients (normozoospermic, asthenozoospermic, oligozoospermic and oligoasthenozoospermic) and fertile men were compared in Lectin-ELISA between O-specific plant lectins (Maclura pomifera and Jacalin) and human Galectin-3. The ligands of Gal-3 were isolated with affinity chromatography. MS identification of Gal-3 ligands was performed after tryptic digestion. Analysis of N- and O- glycans from isolated ligands was acquired in MALDI-TOF MS.

Results and conclusions. No correlation was observed between the reactivity of seminal glycoproteins with O-specific plant lectins and Galectin-3. The reactivity of SP glycoproteins in all analyzed groups did not differ statistically from each other, including the fertile control. To provide a more detailed insight into the glycosylation pattern responsible for Gal-3 reactivity, the normozoospermic group was divided into quartiles based on Gal-3 reactivity, with the two extremes designated for further analysis: low-reactive (Q1) and high-reactive (Q4). Electrophoretic analysis revealed no differences in the protein patterns of isolates from groups Q1 and Q4. Ligands of Gal-3 were identified as lactotransferrin, semenogelin 1 and 2, PIP, PAP, PSA, and clusterin. Gal-3 ligands were shown to include both N- and O-glycans, the vast majority of which lacked sialic acid. Significant differences in the glycosylation profiles were observed between groups Q1 and Q4.

PS4

The effect of metformin on the *N*-glycan profile of the intestinal epithelial barrier *in vitro*.

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Background. The intestinal epithelium is one of the most glycosylated tissues in the body, acting as a barrier that regulates, among other things, digestive processes, absorption, and interactions with microbiota, and its disorders can lead to serious health consequences. It is also known that metformin, a drug used in the treatment of type 2 diabetes, strengthens the integrity of the intestinal barrier by reducing its permeability. In addition, modifications in N-glycosylation can affect cell adhesion, which highlights their importance for maintaining normal intestinal epithelial function. Therefore, the aim of this study was to investigate the effect of metformin on the N-glycan profile of the intestinal epithelial barrier *in vitro*.

Methodology. The research model was based on co-culturing CaCo-2 and HT29-MTX cell lines in a 9:1 ratio, differentiating into enterocytes and goblet cells respectively, that mimic human intestinal epithelium *in vitro*. Epithelial integrity was assessed by measuring TEER. The expression of genes and enzymes related to the *N*-glycosylation pathway was studied using qPCR and Western Blot techniques. The *N*-glycan profile was analyzed using MALDI-MS with a sialic acid linkage-specific derivatization method.

Results and conclusions. We have demonstrated that metformin causes a remodeling of the N-glycan profile of intestinal epithelia. The results indicate a higher relative abundance of oligomannose type N-glycans in cells treated with metformin. Therefore, metformin induces changes in the *N*-glycome of the intestinal epithelium, which may have an important role in regulating the function and maintaining the integrity of the intestinal barrier.

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