

different compartments of the yeasts, including intracellular. Using chromatography and mass-spectrometry the steroids were also found to undergo partial conversion into 3-O-acetates by *S. cerevisiae*, but not *Y. lipolytica*, which was in accordance with computational BLAST search of *S. cerevisiae* Atf2p homologues. Also the compounds' binding with mammalian STARD1 protein were observed. Compound 2 is also promising as a Raman probe due to alkyne insertion into its side chain. Basing on both the results and Autodock Vina-aided docking simulations with various sterol-binding proteins, e.g. STARD1 (3pl0), Osh4 (1zhw), Osh1 (5wvr) and Npc1 (3gkj), we speculate about possibilities of 20-hydroxycholesterol-like compounds to affect yeast cells or mammalian mitochondria as well as future perspectives of such compounds synthesis and usage for studies of 20-oxysterol trafficking and signaling. This work was supported by joint BRFFI (X16P-065) - RFFI (16-54-00139) grant, Belarusian SPSI grant 20161380 and RSF grant 17-74-10053.

#### P.14-017-Tue

##### Characterization of the GM1 oligosaccharide transport across the blood-brain-barrier

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Ganglioside GM1 has demonstrated to attenuate Parkinson Disease (PD) symptoms in clinical and preclinical trials. Nevertheless, the GM1 efficacy revealed *in vitro* is critically reduced *in vivo*, because of the amphiphilic behavior that limits the passage across the blood brain barrier (BBB). *In vitro* and *in vivo* experiments showed that GM1 exerts neurotrophic functions by interacting with plasma membrane (PM) proteins throughout its oligosaccharide portion (OligoGM1). Furthermore, OligoGM1 intravenously or subcutaneously injected into mice is absorbed and taken up by different organs and tissues, including brain. In order to take advantage of GM1 oligosaccharide properties and to overcome GM1 pharmacological limitation, this study has been aimed by the investigation of the OligoGM1 transport through the BBB, by using a human *in vitro* model for human brain-like endothelial cells (hBLEC). Ruled out the toxicity of OligoGM1 on hBLEC, the OligoGM1 transport across the hBBB has been analyzed, finding out a 20 fold higher rate than GM1 and a time and concentration dependence. In order to characterize the OligoGM1 passage, a direct evaluation of the OligoGM1 interaction with the ABC-transporters was carried on, leaving out this way for OligoGM1 transport. Moreover, inverse- and 4°C-transport experiments were performed excluding the implication of the active transport for OligoGM1 passage across the hBLEC, leading to consider the passive-paracellular route. Furthermore, after the hBLEC transport, OligoGM1 maintained its stability and capacity to induce neurogenesis in the mouse neuroblastoma cells line Neuro2a. This preliminary study has improved the knowledge about the GM1 pharmacological potential by proving that OligoGM1 can cross advantageously the BBB, offering a new promising therapeutic strategy.

#### P.14-018-Wed

##### GM1 neuroprotective properties are related to GM1 oligosaccharide

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In light of its neurotrophic and neuroprotective properties, GM1 ganglioside has been considered as a master regulator of the nervous system. Recently, we demonstrated in a mouse neuroblastoma cell line N2a that the oligosaccharide portion of GM1 (OligoGM1) is responsible for the ability of GM1 to induce neurogenesis by the activation of the TrkA-MAPK pathway. This means that the specific role of GM1 in neuronal differentiation is determined by a direct interaction between its oligosaccharide portion and specific proteins expressed on the plasma membrane. In order to understand if the exogenous administration of OligoGM1 and the resulting activation of TrkA pathway is able to trigger crucial biochemical signaling, we performed a proteomic analysis on N2a cells treated with 50 µM OligoGM1 for 24 h. The analysis led to the identification and quantification of 744 proteins; 324 proteins were identified only in OligoGM1-treated cells. Interestingly, the OligoGM1-only proteins are mainly involved in biochemical mechanisms, many of which offer neuroprotective potential reflecting the GM1 neuroprotective effect. To confirm that the neuroprotective effect of GM1 is due to its oligosaccharide portion, OligoGM1-treated N2a cells were administered with MPTP for 48 h. We found that MPTP-induced mortality was reduced by 50% in OligoGM1-treated cells respect to untreated ones. Furthermore, we saw that the administration to N2a cells of dichlorobenzamil (DCB), a potent inhibitor of the Na<sup>+</sup>-Ca<sup>2+</sup> antiporter, which results in elevating intracellular Ca<sup>2+</sup>, is responsible for cell death after 24-h exposure. In presence of 50 µM OligoGM1 the DCB-cell mortality is definitely reduced, suggesting that the modulation of Ca<sup>2+</sup> flux might be one mechanism in the neuroprotective effect due to exogenous administration of OligoGM1. Our results suggest that the molecular mechanisms underlying the neuroprotection effects induced by GM1 depend on its oligosaccharide chain.

#### P.14-019-Mon

##### The subcellular localization mechanisms of type II membrane proteins

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Type II membrane proteins are inserted into endoplasmic reticulum (ER) at the early stage of protein subcellular localization due to the recognition of the signal-anchors by ER translocons. However, the evidential transport mechanisms of transmembrane protein localization from ER to the Golgi or plasma membrane have not been elucidated. Understanding the mechanism of protein subcellular localization is believed to be crucial for control of protein subcellular localization. In this study, to elucidate transport mechanisms of transmembrane proteins, the amino acid propensities around the signal-anchor and C-terminus regions were calculated. The transmembrane protein dataset was classified into three groups: plasma membrane proteins, ER membrane proteins and Golgi membrane proteins. Especially, the trans-Golgi membrane protein dataset was extracted from the Golgi group. The discrimination accuracy of each group was estimated by the discrimination scores which were calculated by the position-specific scoring matrix (PSSM) to evaluate whether the transmembrane protein localization is determined by the