26G-ISMS15 **Functional Characterization of Acetylcholine Receptors in Human Hippocampus-Derived Neurons**

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Utilizing human cell models mimicking physiologically- and/or disease-relevant conditions is a key strategy to improve success rates in drug development. To apply the strategy to the CNS drug development, it is essential to obtain functional human neurons. As a tool to generate functional human neurons, we utilized human hippocampus-derived neural stem/progenitor cells (HIP-009 cells). We previously demonstrated that HIP-009 cells can differentiate into neurons expressing glutamate receptors functionally, and that they were applicable to assessment system of drug candidates for glutamate receptor-related neurological disorders including Alzheimer's disease (AD). In addition to glutamate receptors, acetylcholine receptors (AChRs) are another target group for AD therapeutics. Here, we examined the potential to apply HIP-009 neurons to functional assessment of drug candidates targeting AChRs. qRT-PCR analysis revealed that expression of nicotinic (nAChR) and muscarinic acetylcholine receptor (mAChR)-related genes was upregulated after four-week differentiation. Ca^{2+} flux assays of HIP-009 neurons demonstrated that intracellular Ca^{2+} signals were increased by nicotine and muscarine, indicating that HIP-009 neurons expressed nAChRs and mAChRs functionally. We also pharmacologically investigated expression of each subtype by using subtype-selective compounds, and identified functional expression of $\alpha 7$ and $\alpha 4\beta 2$ nAChRs, as well as M_1 and M_3 mAChRs. These results suggest that HIP-009 neurons are applicable to functional evaluation of drug candidates acting on AChRs for AD drug development.