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MEETING ABSTRACT

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Development and characterization of a human Rett syndrome cell model using a transient non-integrating reprogramming strategy

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Background: Rett syndrome (RTT) is an X-linked progressive neurodevelopmental disorder caused by mutations in the human methyl-CpG-binding protein 2 gene *MeCP2* encoding methyl-CpG-binding protein 2 (MeCP2), a transcriptional regulator possessing both activation and repression properties. MeCP2 is essential for normal brain function and is particularly involved in the maturation and maintenance of neurons. Several studies showed that the lack of MeCP2 leads to impairments in dendritic arborization, electrophysiological properties and neurotransmitter release of neurons. RTT is characterized by an apparently normal development in early childhood followed by developmental stagnation and loss of acquired language, motor and social skills as well as seizures and hypotonia. The purpose of our study is the establishment of a patient-derived neuronal cell model for RTT via a non-integrating reprogramming strategy.

Methods: Human fibroblasts from a MeCP2-deficient patient and a healthy wild type were transfected with two episomal plasmids which encode for the transcription factors PAX6 and SOX2. Transfected cells were cultured in reprogramming medium for several weeks, resulting in induced neuronal progenitor cells (iNPs), which are in turn differentiated into neurons using a two-step protocol [1]. Changes in morphology and mRNA expression levels are monitored through the reprogramming process. The resultant neurons are characterized by immunocytochemical analysis and patch clamp. Moreover, RNA sequencing was performed on MeCP2-deficient and healthy wild-type fibroblasts, iNPs and neurons.

Results: iNPs showed elevated expression of PAX6 and SOX2 in addition to neuronal progenitor associated genes as nestin, neurogenin-2 or FOXG1. Differentiated neurons exhibited typical neuronal morphology and the expression of the neuronal-lineage markers TUJ1, NeuN and MAP2 as well as glutamatergic (vGLUT) and GABAergic (GAD65/67) markers. Furthermore, patch-clamp experiments demonstrated electrophysiological properties of functionally active neurons showing voltage-dependent sodium and potassium channels. RNA sequencing confirmed the presence of neuronal cells and also differences in gene expression are observed between MeCP2-deficient cells compared with a healthy wild type.

Discussion: At present, no efficient therapy is able to change the illness course. One therapeutic strategy would be a protein replacement therapy using a functional MeCP2 fusion protein [2]. This patient-derived cell model for RTT could serve as a promising tool for investigating phenotype rescue through the delivery of a recombinant MeCP2 protein.

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Keywords: cell reprogramming – induced neuronal progenitor cells – stem cell research – protein replacement therapy

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