Reading the Early Signs

Hinz, L.

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SUMMARY

Researchers including Dr. Andreas Rett, Dr. Bengt Hagberg, Prof. Dr. Huda Yahya Zoghbi, and many others improved the life of Rett Syndrome (RTT) patients by investigating the disease mechanisms over the last decades. However, we still have not fully understood all the underlying processes during the appearance of RTT. To understand the onset of RTT, current research focuses specifically on the early neuronal development of RTT patients. Therefore, the usage of induced pluripotent stem cells (iPSCs) has been a crucial approach in investigating prenatal development and observing potential developmental alterations. This technique provides the possibility to study complex pathways and disease onset mechanisms, which might be the key to fully understand RTT. The present thesis aimed to provide new scientific insight into RTT disease onset and the underlying disease mechanisms by studying early neuronal development.

Chapter 1 gives a fundamental introduction about RTT and its history, starting in the early 195Os when Andreas Rett observed the first RTT patients in his doctor's office, up to recent case numbers worldwide. Furthermore, it summarizes early human brain development *in vivo* and how the usage of iPSC technology is capable to study neurodevelopmental processes *in vitro*. Therefore, it creates the basis of our research and explains the background of our disease model.

In **Chapter 2** we investigated the neuronal phenotype observed in primary tissue from adolescent RTT patients and age-matched controls. Specifically, we looked into differences in chloride homeostasis by comparing Potassium-Chloride-Co-Transporter 2 gene (*KCC2)* expression levels. We confirmed previous findings describing a reduction of *KCC2* expression in RTT neurons by investigating *post mortem* brain tissue of RTT patients and age- and gender-matched controls. We suggested, that the lack of KCC2 implements an insufficient shift of γ aminobutyric acid (GABA), from excitatory in juvenile neurons towards inhibitory in mature neurons. This, eventually influences synaptogenesis and is responsible for an imbalance in excitation and inhibition (E/I). Additionally, we showed that in particular one isoform, namely KCC2a was reduced in RTT patients' neurons. As this isoform is predominantly expressed in neuronal cells in the brain stem, it is involved in the development and maintenance of essential physiological processes such as respiration. Even though our findings need follow up studies to completely understand how KCC2a is affecting the development of RTT patients, they postulate a potential mechanism explaining the delay in neuronal maturation and altered activity patterns as described in chapter 4.

Chapter 3 describes the detailed development of the isogenic *in vitro* disease model for X-Chromosomal diseases. It explains the approach to sort RTT patient-derived fibroblasts, to generate pure populations of cells harbouring or not harbouring the *MECP2* mutation. Here, we outlined the use of a specific reprogramming method to generate female iPSCs without reactivating the former inactivated X-Chromosome. This approach leads to the generation of pure iPSC lines from the same donor, being either affected or not-affected by a X-Chromosomal mutation. The generation of such isogenic disease models is very important for the investigation of X-Chromosomal diseases such as RTT. Due to the combination of healthy and affected cells in the sample, altered mechanisms can be overseen due to a low signal to noise ratio. This chapter describes the basic scientific model which is used for all *in vitro* research in the remaining chapters of this thesis.

In **Chapter 4** we investigated alterations in juvenile cortical RTT neurons. We differentiated the generated iPSC lines further and compared activity patterns which appeared during early neuronal development from RTT and control neurons. By performing calcium imaging, we were able to show that the generated RTT neurons have a delay in firing synchronous, which indicates an impaired development of functional circuits. Furthermore, we detected a decrease in the firing frequency of juvenile RTT neurons, which is essential for physiological synaptogenesis and proper neuronal maturation. Based on these findings, we suggested that the altered activity pattern of the generated juvenile RTT neurons could be a potential cause in the underdeveloped phenotype of adult RTT neurons, which also indicates an early, prenatal disease onset.

In **Chapter 5** we investigated quantitative alterations during very early neuronal development. To this end, we differentiated iPSCs towards neuronal stem cells and performed mass spectrometry. We detected alterations in RTT neuronal stem cells already at very early stages. These alterations increased during differentiation towards neuronal stem (NES) cells. In gene ontology (GO) term analysis, we showed that proteins associated with common phenotypic alteration such as *'insulin receptor signalling pathway'*, *'axonal guidance'*, *'cytoskeleton organization'* were already affected at this stage. This implements a prenatal disease onset with alterations adding up until the first appearance of symptoms.

In **Chapter 6** we discussed our previous findings in a bigger context and looked into parallels with other forms of RTT, but also other neurodevelopmental disorders. By identifying overlaps in relevant mechanisms and pathways, we indicated a general issue during neurogenesis. We linked our findings with common pathways involved in

neurogenesis, such as the contribution of IGF-1 or the mTOR pathway. Conclusively, this chapter emphasizes the main findings of this thesis, the onset of RTT before clinical symptoms are manifested, and the potential involvement of elementary developmental pathways.