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From neural induction towards disease models for Tuberous sclerosis complex using human stem cells

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2018

document version
Publisher's PDF, also known as Version of record

Link to publication in VU Research Portal

Summary

Studying defective mechanisms underlying human neurodevelopmental disorders requires appropriate disease models. Human induced pluripotent stem cells (iPSCs) provide new tools to study pathological mechanisms underlying certain diseases or patient populations, with the final goal to improve therapy. The aim of this thesis was to assess the potential of human iPSCs in modeling a specific neurodevelopmental disorder called Tuberous sclerosis complex (TSC), which is placed under Autism spectrum disorders (ASDs). Towards developing proper TSC disease models, we studied several iPSC differentiation protocols for generating specific neural progenitors (NPs) and mature neural cell types, as well as the involvement of brain cell types in TSC phenotypes.

In **chapter 2** we studied the effects of multiple neural induction factors, like dual SMAD inhibition, single SMAD inhibition and retinoic acid (RA) administration, on the regional identity of human PSC-derived NPs *in vitro*. We furthermore assessed the potential of these NPs to commit neuronal and / or astrocytic lineages. Dual SMAD inhibition-induced NPs showed higher expression of glutamatergic neuronal lineage markers, whereas RA-induced NPs showed higher expression of GABAergic neuronal lineage markers. Single SMAD inhibition-induced NPs were less successful in generating cortical neurons, but were efficient in generating astrocytes. We concluded that the tested protocols generate NP populations with similar broadly used marker expression, although they differ in regional identity and differentiation potential.

In **chapter 3** we generated new cortical neuronal differentiation protocols, suitable to study neuron- and astrocyte-specific changes. We showed that these assays produced neuronal networks with balanced expression of GABAergic and glutamatergic synapses. Analysis of protein and mRNA expression at multiple differentiation stages showed expression of cortical neuronal lineage markers like CTIP2, SATB2, PROX1 and MEIS2. Functional assays like calcium imaging and patch clamping in presence of GABAergic and glutamatergic channel blockers AP5/DNQX and bicuculline,

respectively confirmed presence of both types of neurons. Furthermore, the indirect contact cultures showed presence of pure neuronal populations as indicated by proteomic analysis. We concluded that the derived cortical neuronal cultures express a balanced network of cortical excitatory and inhibitory cells. Moreover, this model under indirect co-culture mode would also be useful to study for instance, patient neuron *versus* patient astrocyte effects.

In **chapters 4 and 5**, we studied TSC-patient iPSC-derived neuronal mono-cultures and co-culture networks of patient iPSC-derived neurons and oligodendrocytes (OLs). TSC patient iPSCs showed reduced expression of TSC proteins. While no morphological changes were observed, TSC patient iPSC-derived neuronal mono-cultures expressed increased network activity, which was modulated by administration of clinically used drug Rapamycin. Interestingly, TSC patient neuron-OL co-cultures showed significant changes in axonal density as well as hypertrophy. Furthermore, OL proliferation was significantly increased and OL maturation was reduced in TSC neuron-OL co-cultures. These alterations were also altered or corrected by treatment with Rapamycin. Overall, we concluded that TSC-patient iPSC derived cells possess promising potential to model phenotypic defects of TSC and also respond to pharmacological drugs in a laboratory model. In chapter 5, we assessed long-term survival of iPSC-derived cells in a mouse brain *in vivo* environment. The transplanted iPSC-derived cortical neurons incorporated well into the host brain and expressed GABAergic and glutamatergic markers *in vivo*. Next, we analyzed proliferation and morphological properties of transplanted TSC patient iPSC-derived neurons and OLs. Interestingly, similar to our *in vitro* results, the *in vivo* transplanted cells also showed increased OL proliferation and neurite length. This indicated that future studies with TSC patient iPSC-derived *in vivo* models could serve as humanized mouse models to study TSC phenotypes and help the process of therapy and drug testing.

Finally in **chapter 6,** an overview of the contributions of this thesis, in comparison to current updates in the field are presented. Finally, future prospects of stem cell research towards a better therapy for neurodevelopmental disorders like TSC are discussed.