# New mouse and human Huntington disease iPSC cellular models for use in an experimental cell therapy and for research on neurodevelopmental functions of normal and mutant huntingtin

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Huntington disease (HD) is a monogenic, dominantly inherited neurodegenerative disorder. HD onset usually occurs in adulthood; however, HD can appear in youth or children in its rare juvenile form. The clinical picture of HD include motor, cognitive and psychiatric symptoms. Death of striatal medium spiny neurons is the main neuropathological manifestation of the disease. The cause of HD is a CAG repeat expansion within exon 1 of HTT gene, which results in expression of a toxic huntingtin protein with an extended polyglutamine (polyQ) domain. The disease develops when the number of repeats exceeds 36-40. Huntingtin is the multifunctional protein involved in processes such as transcription regulation, transport and mitotic division. Therefore, the huntingtin mutation results in a wide spectrum of molecular defects in cell functioning. Although multiple cellular and animal HD models have considerably increased understanding of the disease, a successful therapy has not been developed yet.

An autologous cell therapy is a potential therapeutic strategy for HD. In such a therapy, cells derived from a patient would be treated *ex vivo* and transplanted into affected brain regions of the same person. Grafted cells would develop into functional neurons executing regenerative and protective roles. Silencing of the mutant, pathogenic huntingtin expression seems to be an optimal therapeutic strategy for the treatment of autologous cells. Additionally, an induced pluripotent stem cell (iPSC) technology permits establishment of patient-derived cells with a potency to be turned into any cell type of a body.

One of research aims included in this thesis was to create a cellular model for an experimental HD cell therapy in mice. Therefore, I have established and characterized induced pluripotent stem cells (iPSCs) from a YAC128 mouse model of HD. Subsequently, I have stably introduced piggyBac transposon-driven shRNA reagents into the YAC128 iPSC, with the aim of permanent silencing of mutant huntingtin expression. One of the reagents, shHTT2, reduces mutant huntingtin expression by about 85% in iPSC lines and by about 60% in neural stem cells (NSCs) derived from the iPSC lines. The established NSC can be used for an *in vivo* cell therapy of YAC128 mice or for *in vitro* models of a cell therapy.

I have also introduced the shRNA reagents into human iPSCs derived from both HD patients (HD71, HD 109) and healthy people. The potent shHTT2 reagent efficiently silences normal and mutant huntingtin expression in these cells. These are the first human iPSCs with stable knock down of normal huntingtin, which makes them a valuable tool for research on developmental functions of huntingtin in humans.

Huntington disease is traditionally considered as a neurodegenerative disease of an adult brain. However, research from recent years suggests that defects acquired during neurodevelopment are a considerable part of HD pathogenesis. The evidence for the neurodevelopmental phase impact comes from clinical research, as well as from studies in animal models and in pluripotent cell-derived models. During a search for an early biomarker of therapeutic efficiacy in pluripotent cells, I identified deregulated expression of various proteins in HD iPSCs. The observed changes included effectors of Wnt and MAPK/ERK signaling pathways, oxidative stress response proteins and, most importantly, p53 protein. The p53 protein was downregulated both in YAC128-iPSCs and human HD109 cells,

and the mutant huntingtin silencing reversed the phenotype in YAC128-iPSCs. Thus, p53 potentially might be a biomarker for HD therapy in pluripotent cells. The questions on biological relevance of the observed phenotypes remain opened and will be a subject of further research based on results obtained in the derived iPSC models.