MECHANISTIC STUDIES ON PARKINSONIAN NEURODEGENERATION IN LUHMES CELLS - A HUMAN IN VITRO MODEL

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Introduction: Many symptoms of Parkinson's disease (PD) are caused by a progressive loss of dopaminergic neurons in the substantia nigra. As degeneration processes run slowly and single cells are hard to track in vivo, the demand for in vitro neurodegeneration models for mechanistic studies and the discovery of new pharmacological targets is growing.

Aims: PD-like neurodegeneration mechanisms induced by both genetic and toxic factors were studied in a human in vitro model system using LUHMES cells, since these cells possess classical features of in vivo dopaminergic neurons.

Methods: LUHMES were characterized using RT-qPCR, PCR array technology, immunofluorescence, immunoblotting and functional assays. siRNA and lentiviral systems were used for genetic manipulations. Degeneration was induced by the parkinsonian toxin 1-methyl-4-phenylpyridinium (MPP⁺).

Results: LUHMES cells developed a strong dopaminergic phenotype following six days of differentiation. A complex neurite network was formed and dopaminergic markers were upregulated (dopamine transporter, tyrosine hydroxylase, DOPA-decarboxylase, dopamine receptor 2, etc.). Dopamine levels increased, accompanied by susceptibility to low micromolar concentrations of MPP⁺. Several substances, like ascorbic acid and CEP1347, were found to protect from MPP⁺-induced neurodegeneration and are currently used for mechanistic studies on cell death pathways and to study the contribution of genetic components, especially α-synuclein (ASYN), to dopamine-dependent MPP⁺ toxicity. First experiments showed that siRNA-based knockdown of ASYN led to decreased MPP⁺ toxicity and lentiviral overexpression of ASYN induced the formation of aggregates in neurites.

Conclusions: LUHMES cells represent a suitable in vitro model to study both genetic and toxic factors of parkinsonian neurodegeneration.