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Abstract: Charakterisierung von Teratomcybriden mit der primären LHON-Mutation G11778A

LHON, a cell-specific mitochondriopathy, is characterized by an abrupt and fast progressing loss of retinal ganglion cells (RGC), which leads to the degeneration of the optic nerve and results in visual impairment or complete blindness. The primary causes are maternally inherited point-mutations of the mitochondrial DNA, which lead to amino acid exchanges in complex I of the electron-transport chain (ETC). Nuclear modifying genes, ethanol and other noxes are controversially discussed as other factors, because of the incomplete expression and male prevalence of LHON. Although a lot of cell culture studies were performed, the precise biochemical mechanism and the mode of cell death induction by LHON mutations remain enigmatic. Several hints indicate that a synergy of energy deficiency (ATP-depletion) and increased oxidative stress may occur. In the past only one transmitochondrial cell-culture model, based on the osteosarcoma-line 143B.TK(-), was used to determine the mechanism of LHON. In the present thesis an alternative cybrid-model (NT2/D1-teratoma) was utilized, offering the advantage of neuronal differentiation of the clones.
One aspect comprised the consequences of metabolic stress, induced through incubation of cells in galactose medium or differentiation with retinoic acid (RA), to detect the mutation effects. The mutants showed in comparison to wildtype no decreased survival rates. Differentiation with retinoic acid did not lead to a predominant dependency of energy metabolism to the respiratory chain. Furthermore there was no significant decrease of respiration rates with complex-I substrates and decreased ATP-levels in mutants, which was found in the 143B.TK(-)-model. Based on classic bioenergetic parameters no mutational consequences were detectable. In accordance with the hypothesis of a higher saturated antioxidative defence in mutants, the cellular reaction upon external oxidative stress was determined with a cell-survival test. In contrast to theoretic expectations, proliferating mutant cells were less sensitive against H_2O_2, a result which was inversed after treatment with RA. Simultaneously, proliferating cells showed a significantly ($p \leq 0,05$) increased caspase-3 activation of mutants after treatment with this oxidant, indicating that the cell death, measured via cell-survival tests, was probably only partially apoptotic. Despite remaining ambiguities, these findings support the theory of a higher saturation of the antioxidative defence mechanisms in mutants, particularly in context with recently published data of a cooperating partner. The superoxide generating substances paraquat and benzylviologene showed generally low effects, without any correlation to the mutation-status. The same was valid for the noxes ethanol and methanol. Even the reaction of classic triggers of the intrinsic and extrinsic apoptotic pathway (staurosporine and TRAIL) was very low, in contrast to the behaviour of reference cells (143B.TK(-) with common deletion). There was no correlation with the mutation. Measurement of activity of the antioxidative enzymes superoxide dismutase, glutathione peroxidase and glutathione reductase showed, in contrast to former results, no decreased enzyme activity in mutants, even not following differentiation with RA. After differentiation, a mutation specific decreased level of glutathione ($p \leq 0,05$) was measured. The partly controversial results in this thesis in comparison to the up to now used LHON-models indicate, that the cybrid-cell type may have an essential influence on the consequences of the G11778A-mutation with respect to the oxidative energy metabolism and to the cell death under metabolic or oxidative stress-conditions. For this reason, the gained results, based on cybrid cultures, and the possibly resulting pharmacological strategies should not be unconditionally transferred to retinal ganglion cells.