Identifying isoform- specific Metallothionein expression in Rotenone induced NADH:ubiquinone oxidoreductase deficiency in HeLa cells

Y Olivier, F Reinecke, O Levanets, T Semete, R Louw, A Olckers and FH van der Westhuizen.

Division of Biochemistry, School for Chemistry and Biochemistry, North-West University, Potchefstroom 2520.

Some well defined functions of crucial importance for cell physiology are carried out by NADH:ubiquinone oxidoreductase (complex I). Deficiencies of complex I leads to multi-system disorders that includes diabetes, Alzheimers disease and MELAS to name only a few. Metallothioneins (MTs), which are ROS-sensitive proteins, were recently identified to be overexpressed in complex I deficiency although the role that the different isoforms play still remains unclear. We investigated the expression of specific metallothionein isoforms type 1 A (MT-1A) and B (MT-1B) as well as type 2 (MT-2) in rotenone induced complex I deficient HeLa cells, using real-time PCR and ELISA. Five different housekeeping genes (GAPDH, β-actin, β-2-microglobulin, RNA polymerase II and 18S rRNA) have also been tested for their suitability to be use as internal controls for normalisation, also testing stability in the presence of metals- (Cadmium and Zinc) and ROS induction (*tert*-Butyl hydroperoxide). The transcriptional response of MT-2 shows an increase of more than 6 times at the highest rotenone induction after 24 hours, as compared to baseline levels. Furthermore, we show data of increased production of ROS for rotenone and *t*-BHP. We hypothesized that with a complex I deficiency, an increase in ROS production will ultimately lead to an increase of MT expression and that this underline the importance of nucleus-mitochondrial communications as a response to mitochondrial disease.