Functional properties of Metallothionein overexpression in rotenone-induced NADH:ubiquinone oxidoreductase-deficient HeLa cells.

[1]**F.Reinecke**, [1]O. Levanets, [1]R. Louw, [2]B. Semete, [1]Y. Olivier, [2]A. Olckers and [1]F.H. van der Westhuizen

[1]Division of Biochemistry, School for Chemistry and Biochemistry, [2]Centre for Genome Research North-West University, Potchefstroom, 2520.

Present data show that deficiencies of inherited mitochondrial energy metabolism have a prevalence of approximately 1:8 500 amongst neonates, indicating they are some of the most common forms of metabolic disorder. Due to their debilitating consequences, current investigations into potential protective agents against these deficiencies are becoming more important. NADH:ubiquinone oxidoreductase (Complex I) deficiencies have been known to result in, amongst others, high levels of reactive oxygen species (ROS). These lead to various consequences, including the induction of apoptosis. Metallothioneins (MTs) are a family of metal-binding proteins with unique structural characteristics providing them the ability to bind and reduce ROS, as well as metals. To unveil the role of MTs in complex I deficient cells, we overexpressed MT-1B and MT-2A in HeLa cells. Their cDNAs were incorporated into pIRESneo2 vectors and transfected into HeLa cells. The expression of the MTs was confirmed with Northern Blots. Rotenone-induced complex I deficient cells were analysed for ROS production and apoptosis induction in a dose-dependant manner. MT-1B and especially MT-2A over-expression significantly decreased caspase 3/7 activity and DNA fragmentation, indicating protection against rotenone-induced apoptosis. In addition, lower levels of ROS and increased cell viability occurred and mitochondrial membrane potential integrity was also protected in the cell lines. The importance of MT-2A expression is more significant than MT-1B because the latter has no or very low expression levels in HeLa cells under normal conditions. In light of this data and previous reports, metallothioneins seem to be a good candidate for gene-expression targeted therapeutic intervention.