Expression of an external alternative NADH:ubiquinone oxidoreductase of *Aspergillus fumigatus* in human cell

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**Introduction:** Mitochondrial respiratory chain defects are found in several diseases such as cancer, aging and degenerative diseases. However, the mechanism or pathophysiology of several of these dysfunctions remains unclear. Biochemical and molecular studies of *Aspergillus fumigatus* in our laboratory showed the presence of alternative enzymes in the mitochondrial respiratory chain. One these enzymes is NADH:ubiquinone oxidoreductase (Nde) which allow an alternative entry of NADH in the respiratory chain. In this sense, expression of Nde is a resource to understand the variation of NAD+/NADH ratio in human cells.

**Objective:** The objective of this study was to obtain the condition of overexpression of Nde in human embryo kidney (HEK293) cell.

**Material and Methods:** For this purpose the Nde cDNA was cloned into pGEM-T easy vector (Promega). Then, it was cloned into Gateway pDNOR/Zeo vector (Life Technologies) and the recombinant reaction was performed with pcDNA 3.1 Mammalian Expression vector (Life Technologies). In all the steps the sequence was confirmed by DNA sequencing. The transfection into HEK293 cells was performed using 1, 2, 3 or 4 µg of expression plasmid and Polyfect Transfection Reagent (QIAGEN). The expression of Nde was monitored 24, 48 and 72 hours after transfection.

**Results and discussion:** The best condition of expression of Nde in HEK293 cells was 48 hours after transfection using 2 µg of expression plasmid. The expression of Nde was confirmed by confocal microscopy and western blotting using anti-V5-tag antibody.

**Conclusion:** The expression of Nde in human cell will enable us to use Nde to study mitochondrial diseases which promotes changes in the ratio of NAD+/NADH.

Keywords: mitochondrial disease, Nde, HEK293