

## Heterologous Expression of Nicotinamide Adenine Dinucleotide Gene from *Aspergillus fumigatus* in HEK293 Cells.

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**INTRODUCTION.** *Aspergillus fumigatus* is a saprophytic species, being one of the main pathogens in immunocompromised patients, causing aspergillosis. Mitochondria of *A. fumigatus* possess classical and alternative electron transport chain. In addition, they present a nicotinamide adenine dinucleotide carrier (Ndt1). NAD<sup>+</sup> and its derivatives have a central role in cellular metabolism. Mutations or deletions in oxidative phosphorylation genes have been described as the cause of many degenerative diseases, cancer and aging. Thus, expression of fungi alternative proteins in mammal cells could help us understand cellular metabolism and restore mitochondrial dysfunctions. **OBJECTIVE.** The aim of this study was to clone, sequence, and express *A. fumigatus ndt1* gene in HEK293 cells. **MATERIAL AND METHODS.** A sequence of 1,194 bp was amplified and cloned in pDONR<sup>TM</sup> vector using BP reaction. The sequence was subcloned into pcDNA3.1/nV5-DEST<sup>TM</sup> expression vector. The pcDNA/*ndt1* construction was transfected into HEK293 cells using PolyFect<sup>®</sup> transfection reagent. Stable line was selected using 750 µg/mL geneticin. Expression of recombinant protein was confirmed by western blot using anti-V5 antibody. To confirm the cellular localization of Ndt1 protein, HEK293 cells were subjected to confocal microscopy using fluorescent probes MitoTracker<sup>®</sup>, anti-Ndt1 antibody, Alexa Fluor<sup>®</sup> 488 and DAPI. **DISCUSSION AND RESULTS.** DNA sequencing confirmed that *ndt1* gene was cloned in correct frame and without nucleotides alterations. Ndt1 protein was detected by western blot in stable line after transfection with 2 µg of pcDNA/*ndt1* construction and selection with geneticin. Confocal microscopy evidenced the co-location of Ndt1 protein with mitochondria in HEK293 cells. The same profile was not observed in control line. **CONCLUSION.** In this study, we cloned and sequenced of *ndt1* gene from *A. fumigatus* in a mammal expression vector, and we determined the cellular localization of Ndt1 protein in HEK293 cells by confocal microscopy.

**Keywords:** *A. fumigatus*, HEK293 cells, mitochondria, heterologous expression, Ndt1 protein.

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