Evaluation of carbon nanotubes for delivering peptides into mitochondria

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Mitochondria are found ubiquitously in nucleated eukaryotic cells. One of their key functions is to generate ATP via oxidative phosphorylation, in order to provide the cell with a readily usable form of energy. The machinery responsible for this process comprises 5 complexes, members of which are encoded by either the nuclear or the mitochondrial genome. Energy transduction in the cell is, thus, dependent of the precise and accurate intramitochondrial translation of the 13 mitochondrial (mt)DNA encoded polypeptides. Defects in mitochondrial metabolism are being increasingly recognized as a cause for disease. Indeed, mitochondrial disease is no longer described as a rare disorder, in part as a result of better awareness and therefore more accurate diagnosis. Pathogenesis can arise from defects in either nuclear encoded proteins that function in the mitochondria or from mutations in the mt DNA itself. The relative ease of sequencing the comparatively small mitochondrial genome has allowed a comprehensive characterization of mt DNA mutations associated with mitochondrial disease, establishing that the majority of these pathogenic mutations reside in the mt tRNA genes. These would be predicted to impair mitochondrial protein synthesis and since it is currently not possible to manipulate the human mtDNA, researchers have explored other ways to overcome defects of mt tRNAs. Such approaches have often used yeast as a model system where also has been possible to isolate nuclear suppressor factors able to rescue the defective phenotype of the mutants.

The ability of overexpressed cognate mt aminoacyl-tRNA synthetase (aaRS) to attenuate the detrimental effects of mt tRNA point mutations has been demonstrated both in yeast and human cell lines bearing equivalent tRNA mutations. Recently we have shown that, mt ValRS and LeuRS enzymes (belonging to Class Ia of mt aaRS) rescued the phenotypes determined by mutations in either the cognate or the non-cognate yeast mt tRNA. The suppression activity of the mt LeuRS has been studied in detail and the carboxy-terminal domain (Cterm) of mt LeuRS was identified as is the "key region" necessary and sufficient to rescue the defective phenotype of pathological mutations in several mt $tRNA^{Leu}$, mt $tRNA^{Val}$ and mt $tRNA^{Ile}$ in yeast and in human cells.

Our results, by showing the mitochondrial localization of the Cterm and its specific *in vitro* interaction with mt $tRNA^{Leu(UUR)}$ strongly suggest that the peptide is active into mitochondria.

The ability of the small (67 residues long) Cterm to rescue severe defects associated with cognate and non-cognate mt tRNA mutations opens new perspectives for treatment of human diseases associated with mt tRNA mutations. The main objective of our research is the exploitation of this knowledge to develop therapeutic strategies. To this end we use the yeast *Saccharomyces cerevisiae* as cell model system to deliver peptides derived from mt LeuRS Cterm known to suppress the mt defects to the mt compartment using carbon nanotubes (CNTs) as carrier.