UNIVERSITA' DEGLI STUDI DI PARMA

Ph.D IN BIOTECHNOLOGY AND LIFE SCIENCES, XXXIV CYCLE

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During my first year of Ph.D, I worked on genes associated with Hereditary optic neuropathies (HON). HON are genetic diseases which lead to the loss of central vision and it has been estimated that in Europe they affect more than 50.000 people. HON are characterized by selective loss of retinal ganglion cells (RGCs), leading to optic nerve atrophy and different degrees of visual impairment and blindness.

Thanks to Next Generation Sequencing and Whole Exome Sequencing, three heterozygous mutations in SDHA gene were identified in patients, that could be associated with these pathologies. SDHA encodes for one of the four subunits of the succinate dehydrogenase complex, involved both in the Krebs cycle and in the mitochondrial electron transport chain. To evaluate whether these mutations are the specific cause of the pathology, it was necessary to validate them. The yeast *Saccharomyces cerevisiae* is extensively used to prove with high confidence the link between novel mutations and mitochondrial diseases, since yeast can survive without mitochondrial DNA or with large deletions of it, and can grow on fermentable carbon sources in absence of an oxidative metabolism. Besides, several human genes encoding for mitochondrial proteins are present and often conserved in yeast, giving the chance to introduce the mutation in the yeast orthologous gene or to introduce the human pathological allele in a strain disrupted in its orthologue.

SDHA and *SDH1*, its yeast orthologue, encodes for proteins which share more than 60% of identity, and the three variants found in patients affect amino acids which are

conserved in most organisms, including fungi and mammals. The three mutations (L367P, R444C, R593Q) were introduced in *SDH1*, and these mutant alleles were inserted in a *SDH1*-disrupted strain. Our results showed that the L367P and R444C mutations lead to a decrease of the oxidative growth at 28° on non-fermentable carbon sources, showing the mitochondrial damage caused by these two mutations. The L367P variant at the physiological temperature had similar growth compared to WT. The respiratory rate of the strains harboring these variants was reduced compared to the strain containing the WT allele, demonstrating an impairment of the mitochondrial respiratory chain. For the R444C and R593Q mutations, it was possible to observe a decrease at 28 °, while the L367P mutation affected the phenotype at 37°, and for this reason it was considered thermosensitive. Using mitochondrial extracts, it was possible to evaluate the activity of the SDH complex, through spectrophotometric monitoring of the reduction of a synthetic electron acceptor. The R444C and R593Q mutations led to a large impairment of the complex enzymatic activity, whereas once again the L367P mutation at physiological temperature showed no difference compared to WT allele. The experiment was performed also at 37°, and as in the previous analysis a thermo-sensitivity of this mutation was observed. Western blots showed that the steady state levels of the mutant proteins were similar or slightly reduced compared to the levels of wild type Sdh1, whereas Sdh2, the second subunit of the complex which interacts directly with Sdh1, is absent or strongly decreased. These results suggest that the substitutions in Sdh1/SDHA are pathological and that they affect the stability of the complex. Besides, through the construction of heterozygous diploid strains, we demonstrated that two of three mutations are dominant negative, thus explaining the observation that the patients are heterozygous for each of these mutations.