ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA
DIPARTIMENTO DI SCIENZE MEDICHE VETERINARIE

Title

Structural and functional analysis of NDUFC2-dependent Complex I dysfunction on cardiac mitochondria of spontaneously hypertensive rats.

Project description

Mitochondrial dysfunction dependent on deficiency of Complex I (*CI*, NADH: ubiquinone oxidoreductase) contributes to cardiac hypertrophy in animal models. NDUFC2 subunit deletion causes *CI* disassembly and mitochondrial dysfunction. In this project, the impact of NDUFC2 subunit deletion on the cardiac phenotype associated with hypertension will be investigated to understand the molecular mechanism of *CI* dysfunction underlying impaired mitochondrial function and the hypertrophic phenotype. Two rat models will be used for *in-vitro* and *ex-vivo* studies: the Spontaneously Hypertensive Rat (SHR/*wt*) and the heterozygous *Ndufc2-KO* SHR (SHR/*ndufc2*$^{+/-}$). On cardiac mitochondria from the two models, we will investigate the coupling between the electron transport chain and the ATP synthesis machinery to provide information on the mitochondrial oxidative phosphorylation (OXPHOS) function/dysfunction. The link between the ROS level and the mitochondrial membrane potential (mmp) hormesis, which are related to the respiratory supercomplex (SC) organization will be considered. The mitochondrial bioenergetic parameters, OXPHOS, mmp, ROS production, the proteomics of SCs assembly factors will be compared in mitochondria of SHR/*wt* and SHR/*ndufc2*$^{+/-}$.

Proposal plan

In-vitro studies on mitochondrial suspensions will evaluate the coupling between the membrane electron transfer system (membrane-ETS) and the ATP synthesis machinery. In detail, mitochondrial respiration and/or ATP synthesis will be explored by evaluating the sequential electron flow through respiratory complexes and the oligomycin-sensitive ATP synthase activity. Then, the membrane-ETS will undergo a kinetic test to identify the metabolic flux control analysis. The flux control coefficients of the complexes involved in aerobic NADH oxidation will be detected by titrating the whole respiratory chain activity (global flux) and its single steps with inhibitors of the individual complexes, *i.e.* rotenone *CI*, mucidin for Complex III (*CIII*), and KCN for Complex IV (*CIV*). The role of NDUFC2 subunit as a rate-controlling or rate-limiting step in the metabolic pathway will be indicated by either the presence or absence of substrate channelling toward *CIII* and *CIV*. The kinetic properties of the membrane-ETS will be verified with the assembly of the supramolecular aggregates, comprising the respiratory complexes (supercomplexes, SCs), in relation to the NDUFC2 subunit deletion, based on the alterations observed in SHR/*ndufc2*$^{+/-}$ compared with SHR/*wt*. The extent of aggregation of SCs will be investigated in digitonin-treated mitochondria by electrophoretic analysis (2D BN/SDS PAGE) followed by Western blotting and identification by immunodetection of specific subunits of the individual respiratory complexes. The loss of SCs organisation may be involved in a vicious circle of oxidative stress and energy failure. A direct evaluation of superoxide production will be performed by fluorescence detection in the presence of the redox-sensitive probes MitoSOX, a mitochondrial superoxide indicator, and of the mitochondrial redox status, considering the measurement of redox couples cysteine/cystine (Cys/CySS) and the NADH/NAD$^+$ ratio.

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