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INTEGRATED APPROACHES FOR THE STUDY OF MITOCHONDRIAL DYNAMICS

Center for Neurosciences and Cell Biology, Coimbra,
May 11-15, 2009

Pratical Course (20 students)

Organizers

Anabela Rolo, Carlos Palmeira, João Laranjinha, Ana Ledo, Vilma Sardão.

Session 1: Evaluation of alterations in mitochondrial function associated with hepatic pathology

(Anabela Rolo, Carlos Palmeira, João Laranjinha, Paulo Oliveira)

1.1. Isolation of mitochondrial fraction.
1.2. Evaluation of oxygen consumption.
1.3. Evaluation of mitochondrial membrane potential.
1.4. Evaluation of calcium-induced mitochondrial permeability transition.
1.5. Evaluation of mitochondrial calcium fluxes.

Session 2: Modulation of cytochrome oxidase activity by nitric oxide

(João Laranjinha e Ana Ledo)

2.1. Kinetics of nitric oxide release by NO donors. Electrochemical quantitation.
2.2. Simultaneous evaluation of oxygen and nitric oxide concentration in mitochondrial suspensions.
2.3. Modulation of oxygen consumption by nitric oxide.

Session 3: Drug-induced mitochondrial dysfunction and cell death in cultured cells

(Paulo Oliveira e Vilma Sardão)

3.1. Basic aspects of cell culture.
3.2. Incubation of cells for 24 hours with anti-cancer agents (doxorubicin, berberine).
3.3. Evaluation of apoptosis/necrosis.
3.4. Imaging of mitochondrial physiology by epifluorescence microscopy.
3.5. Extraction of total, cytosolic and mitochondrial extracts from cells in culture.
3.6. Western blotting to evaluate alterations in quantity and location of apoptosis-related proteins.

Session 4: Real-time quantitative RT-PCR analysis of gene expression

(Anabela Rolo)

4.1. Extraction of total RNA from tissue samples.
4.2. Quantitation and RNA analysis using the Experion Automated Electrophoresis System.
4.3. Primer design and cDNA synthesis.
4.3. SYBR Green Dye assay.
4.4. Quantitation with standard curves, data analysis.

Session 5: Translational alterations in proteins that regulate mitochondrial biogenesis

(Carlos Palmeira)

5.1. Extraction of total proteins from tissue samples.
5.2. Immunoprecipitation.
5.3. Quantitation of acetylation status by western blot.

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