POLITECNICO DI MILANO

PhD in CHIMICA INDUSTRIALE E INGEGNERIA CHIMICA / INDUSTRIAL CHEMISTRY AND CHEMICAL ENGINEERING - 39th cycle

PNRR 117 Research Field: DEVELOPMENT OF ARTIFICIAL EXTRA CELLULAR VESICLES TO POTENTIATE MITOCHONDRIAL TRANSPLANTATION

Monthly net income of PhDscholarship (max 36 months)
€ 1400.0 In case of a change of the welfare rates during the three-year period, the amount could be modified.

Context of the research activity	
Motivation and objectives of the research in this field	Mitochondria are cytoplasmic double-membrane organelles (200-1000 nm) that are referred to as the powerhouses of the eukaryotic cell because they are the sites where synthesis of adenosine triphosphate (ATP) associated with aerobic oxidative phosphorylation (OXPHOS) occurs. Mitochondria have their own double-stranded DNA (mtDNA). Each individual mammalian cell contains hundreds or thousands of mitochondria, which in turn contain between 1 and 15 molecules of mtDNA. The ratio of mtDNA molecules/cell varies according to cell and tissue types, and mitochondria exhibit polyplasmy, with the mtDNA genotype resulting either from a single type of mtDNA (homoplasmy) or from the coexistence of multiple mtDNA haplotypes in varying amounts (heteroplasmy). If the proportion of mutant molecules in the mitochondria exceeds a certain threshold, mitochondrial diseases may occur (the so-called "threshold effect"). Mitochondrial diseases (MDs) are inherited genetic diseases characterised by pathogenic mutations in nuclear DNA (nDNA) or mitochondrial DNA (mtDNA).Current therapies are still far from being fully effective and from covering the broad spectrum of mutations in mtDNA. One attractive therapeutic approach for MDs is mitochondrial transplantation to provide dysfunctional cells and/or tissues with healthy mitochondria. This approach could also be potentially broader as mitochondrial dysfunction is

	a known trigger for many diseases.In this project, we will use extracellular vesicles (EVs), nanoscale circular phospholipid structures released into the extracellular space by almost all cell types under both physiological and pathological conditions, as a biological cargo of mitochondria and their components. EVs are considered physiological vehicles with low immunogenicity and toxicity, which enhance the maintenance of mitochondrial integrity during transfer into recipient cells. They are classified into three general classes according to their nanodiameter and biogenesis: (1) exosomes (30-150 nm); (2) microvesicles (150-1000 nm); (3) apoptotic bodies (> 1 µm). Due to the limited size of exosomes, only the microvesicles (MVs) could contain intact mitochondria and contribute to the intercellular transfer of mitochondria.The aims of this project are:- isolate EVs from mouse neuronal progenitor cells, which are then labelled with specific fluorochromes or other imaging probes to allow tracking of EVs in recipient cells *in-vitro*;- using EVs isolated from the mitoQC transgenic mouse model that constitutively express the mCherry-GFP-FIS1 reporter, *i.e.*, a pH-sensitive fluorescent mitochondrial signal, that is a valuable tool for visualizing the fate of mitochondria in the recipient cells;- use a combination of labelled EVs and labelled mitochondria to track their respective fates in the recipient cells;- create artificial EVs able to host isolated mitochondria to be delivered to recipient cells.
Methods and techniques that will be developed and used to carry out the research	This research project will include 1) isolation of mitochondria from cells and tissues and evaluation of their integrity and functionality; 2) isolation of EVs from neuronal progenitor cells. The isolated EVs will be characterized by physico-chemical techniques in terms of size, morphology, and colloidal stability, as well as in terms of their mitochondrial protein content. The EVs will be characterized by nanoparticle tracking analysis, small angle X-ray scattering, and electron microscopy. EVs will be isolated by a differential ultracentrifugation method. Specifically, neuronal progenitor cells will be dissociated to single cells and plated in culture flasks with a specific medium. After 18 hours, the medium will be collected and centrifuged to remove cells and debris. The supernatant

	will then be collected again and subjected to a furtherl ultracentrifugation. The intracellular fate of labelled mitochondria and labelled EVs will be studied *in-vitro* with confocal microscopy. These studies will be done *in-vitro* using different types of cells and *in-vivo* using intra-vitreal injection of EVs in a mouse model carrying a homoplasmic mtDNA variant in the ND6 gene. In detail, we will use:1) neuronal progenitors derived from mouse embryo and from adult brain;2) rho zero cells that are cells devoided of mtDNA in which the transfer of exogenous mitochondria and mtDNA can easily be followed;3) fibroblasts derived from patients carrying different mtDNA mutations;4) neuronal progenitors cells derived from iPSCs generated from patients carrying different mtDNA mutations;In addition, the level of mtDNA heteroplasmy will be evaluated through Next Generation Sequencing technology.Parallely to these studies, artificial giant lipid vesicles will be prepared for hosting isolated mitochondria to be delivered to recipient cells and their delivery ability will be tested and compared to that of EVs. Different modalities will be used to encapsulate healthy mitochondria in these artificial vectors (lipid film hydration, electroporation, etc.).
Educational objectives	•Learn how to isolate and purify EVs •Learn how to prepare and characterize nano-scale materials •Learn cell culture practices •Learn how transplant mitochondria through EVs *in-vitro* and *in-vivo* models •Learn to consider how to transfer research activities into clinical practice
Job opportunities	•R&D positions in biotech and pharmaceutical companies •Biomaterial scientists in chemical and biomedical companies •R&D positions in Drug and Gene Delivery Technology companies •Researcher in IRCCS or similar biomedical research

	institutes/Hospitals •R&D position in biotech company focused on research, development, manufacturing and clinical validation of innovative therapies
Composition of the research group	2 Full Professors 3 Associated Professors 5 Assistant Professors 6 PhD Students
Name of the research directors	Proff. Baldelli Bombelli, Metrangolo

Contacts
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Additional support - Financial aid per PhD student per year (gross amount)	
Housing - Foreign Students	--
Housing - Out-of-town residents (more than 80Km out of Milano)	--

Scholarship Increase for a period abroad	
Amount monthly	700.0 €
By number of months	6

National Operational Program for Research and Innovation	
Company where the candidate will attend the stage (name and brief description)	Fondazione Istituto Neurologico Carlo Besta (FINCB) - IRCCS Via Giovanni Celoria, 11, 20133 Milano MI https://www.istituto-besta.it/
By number of months at the company	6
Institution or company where the candidate will spend the period abroad (name and brief description)	Dept. of Clinical Neurosciences, University of Cambridge Clifford Allbutt Building - Cambridge Biosciences Campus Hills Road, CB2 0AH Cambridge, UK https://www-neurosciences.medschl.cam.ac.uk/
By number of months abroad	6

Additional information: educational activity, teaching assistantship, computer availability, desk availability, any other information
Confidentiality (in case of DM 117 – Agreement with company): since this is a thematic scholarship, the management of Confidential Information, Results and their publication is subordinate to the restrictions agreed upon with the funding company. Upon acceptance of the scholarship, the beneficiary must sign a specific commitment. **Individual budget for research** (5.700 euro):1st year: 1.900 euro; 2nd year: 1.900 euro; 3rd year: 1.900 euro **Teaching assistantship (availability of funding in recognition of supporting teaching activities by the PhD student):** there are various forms of financial for activities of support to the teaching practice. The PhD student is encouraged to take part in these activities within the limits allowed by the regulation.