DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO

41° Cycle

Title of the research: Engineering, Development, and Validation of an EIS-Mediated Exosomal System for the Targeted Delivery of Heterologous miR-25: A Novel Therapeutic Strategy to Promote Endogenous Neurogenesis After Stroke

Supervisor: Sandra Marizabel Columba Cabezas mail: sandra.columbacabezas@iss.it
Dipartimento di Neuroscienze – Istituto Superiore di Sanità

Tutor: Ada Maria Tata mail: adamaria.tata@uniroma1.it

Host Institution: Istituto Superiore di Sanità

Summary
Ischemic stroke (IS) may trigger proliferation, migration towards the ischemic lesion, and differentiation of neuroprogenitor cells into mature neurons. Recent findings demonstrate that miR-25 plays a significant role in neurogenesis mechanisms, as evidenced by its increased plasmatic levels in subacute ischemic stroke patients treated with high-frequency repetitive Transcranial Magnetic Stimulation (HF-rTMS). The study revealed that HF-rTMS significantly elevated plasma levels of miR-25 and Netrin-1, and borderline increases were observed for miR-106b and miR-93, all of which are associated with neurogenic and axonogenic pathways. Notably, HF-rTMS emerged as an independent predictor of higher miR-25 and Ntn-1 levels, even after adjusting for confounding factors. These results underscore the importance of miR-25 as a key molecular player in post-stroke endogenous repair mechanisms and highlight its potential as a promising candidate for nano-drug delivery strategies. By incorporating miR-25 into engineered exosomes or nanoparticle-based systems, it may be possible to enhance neurogenesis and functional recovery in stroke patients, offering a novel therapeutic avenue grounded in personalized and regenerative medicine. Building on these findings, Aim 1 of this project is to develop a nano-therapeutic strategy to enhance neurogenesis and angiogenesis by delivering therapeutic RNAs, such as miR-25 or members of the miR-17~92 cluster, into neural cells using engineered exosomes or nanoparticle-based systems. To improve RNA loading efficiency, we will incorporate exosomal RNA-targeting sequences, such as those described by Cabezas & Federico (2013), where RNA segments from the HIV-1 Gag p17 region were shown to direct selective RNA packaging into exosomes that will be named exosome incorporating sequence (EIS). Following the characterization of the engeenered exosomes, Aim 2, we will establish in vitro cellular models of hypoxia to investigate the functional relationship between these miRNAs and their target proteins (e.g., HMGB1). These models will include neuroblastoma cell lines and neural progenitor cells derived from human induced pluripotent stem cells, subjected to oxygen-glucose deprivation to mimic ischemic conditions. This integrative approach combining biomarker discovery, nanotechnology, and disease modeling holds potential for the development of personalized RNA-based therapeutics that enhance post-stroke regeneration and recovery.